THE CHEMICAL FORMS AND PLANT AVAILABILITY OF COPPER IN COMPOSTING ORGANIC WASTES

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Signature……..

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Abstract

A seven-step sequential extraction scheme was used to track changes in operationally defined copper speciation during the composting of a mixture of grass clippings and sawdust originating from tanalised timber. Starting materials were either unamended or treated with differing amounts of soluble copper, using a copper acetate solution, and then composted in the laboratory. Results showed that at the start of the experiment over 80% of the copper present in the unamended materials occurred in forms not immediately available for plant uptake. However, composting processes enabled the release of this copper which then, over time, became more bioavailable. Large amounts of copper in the copper amended materials were initially detectable in all fractions except the residual one, but over time it was seen to move from all fractions to the EDTA extractable fraction, thought to determine organically complexed / chelatable metals (Amir, 2005). This continued until an equilibrium was reached and then the water and calcium nitrate extractable forms appeared to hold the excess. Copper as determined by these extracts would be available for plant uptake.

In the second experiment, three different organic wastes (grass/sawdust, pig slurry/sawdust and sewage sludge cake/sawdust) to which copper had been added as copper acetate, sulphate or EDTA, were composted in the laboratory. Samples were taken at 0, 105 and 318 days and subjected to a range of analyses: copper by sequential extraction using two different

extraction schemes, a chelating resin membrane (CRM) procedure and by XRF spectrometry; FTIR analysis for functional groups; total carbon, nitrogen and sulphur; pH, EC, NH_4^+ and NO_3^- nitrogen, COD, germination indices and optical properties of water extracts. Sequential extractions demonstrated clear changes in copper distribution amongst various fractions within the materials, with copper originally present in the materials being transferred from the oxidisable fractions to easily extractable (and hence potentially phytoavailable) fractions. Transfer of copper from available to less available fractions in copper amended materials was also seen with movement of copper within copper EDTA treated materials being the slowest of all. Initial amounts of copper in fraction 1 extracted from all samples determined the rate at which copper was transformed. CRM determined copper correlated strongly with copper from fraction 1 of the Tessier scheme, although changes over time did not correspond well. Other parameters measured indicated that that the material was maturing (decreases in C/N and polysaccharide functional groups). However, other results demonstrated that the composts were still immature and unstable. Such slow decomposition was attributed to the high lignin content of the materials. Nevertheless, immobilisation of potentially phytotoxic level of copper was still demonstrated. The usefulness of chelating resin membrane as a predictor of phytoavailable copper is also discussed.

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Abbreviations

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid……HEPES

Analysis of Variance……ANOVA

Atomic Absorption Spectrophotometry……AAS

Bureau Community Reference……BCR

Carbon to Nitrogen Ratio……C/N

Chelating Resin Membrane……CRM

Chemical Oxygen Demand……..COD

Coefficient of Variation……CV

Diethylenetriamine pentaacetic acid……...DTPA

Electrical Conductivity……..EC

Ethylenediamine tetraacetic acid……..……EDTA

Field Capacity……...FC

Fourier Transform Infra-Red Spectrometry……FTIR

Germination Index……..GI

High Density Polyethylene……HDPE

Industrial Methylated Spirits……...IMS

Least Significant Difference……..LSD

Municipal Solid Waste……..MSW

Relative Standard Deviation……% RSD

Waste Resource Action Program……WRAP

X-Ray Fluorescence Spectrometry……..XRF

Sample Treatments in Chapter 3

Untreated grass and sawdust blend…….....UGS

Copper acetate treated grass and sawdust blend.....AGS

Copper sulphate treated grass and sawdust blend.....SGS

Copper EDTA treated grass and sawdust blend.....EGS

Untreated pig slurry and sawdust blend.....UPS

Copper acetate treated pig slurry and sawdust blend.....APS

Untreated sewage sludge cake and sawdust blend.....UCS

Copper acetate treated sewage sludge cake and sawdust blend.....ACS

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Introduction

Waste and its management is an issue of considerable importance in the EU, with England and Wales alone producing around 400 million tonnes of waste are produced every year, the bulk of which goes to landfill (DETR, 2000a). Housed livestock also produces 80 million tonnes of waste and the general public generates a further 35 million tonnes in the form of sewage.

Composting can be an effective way of dealing with biodegradeable wastes and forms part of the UK Government's *'Waste Strategy 2000'* (DETR, 2000a) which seeks to deal with the growing problem of waste production. Not only does composting reduce the volume of material in question but, when conducted and properly, produces an end product which is stable, sanitised and supports plant growth (British Standards Institute, 1994). However, there can be problems if the raw materials are contaminated with heavy metals and/or organic compounds and these will be magnified due to the concentrating effect of the composting process.

Copper is one such heavy metal which can be found in potentially compostable waste streams. At low levels it serves as a plant micronutrient. However, at much higher levels it can become toxic to both plants and microorganisms (Keeling and Cater, 1998). Historically, heavy metal content has focussed on total analysis. Such an approach means that all forms of a given metal are implicitly considered to have an equal

impact on the environment (Martin *et. al.* 1987) and legislation is still based on total metal contents (MAFF, 1998). However, it is now generally recognised that bioavailability is dependant on chemical forms or 'speciation' (Quevauviller *et. al.,* 1997).

This project has sought to determine the changes that copper undergoes during the composting of several different biodegradeable waste materials at the laboratory scale. In the first trial (Chapter 2), grass clippings and sawdust were blended and treated with a range of copper levels. The materials were then composted. Samples were taken at regular intervals during the composting process and analysed for copper using a 7-step sequential extraction scheme which operationally defined copper speciation within the material. In the second trial (Chapter 3), three different wastes – grass clippings, pig slurry and sewage sludge cake – were blended with sawdust, treated with copper at 2 levels (0 and 1000 mg kg^{-1} on a fresh weight basis), composted and sampled at 3 different times. Copper was determined using 4 different approaches – 2 well known sequential extraction schemes, (Tessier *et. al.,* 1979; Ure *et. al.,* 1993), a chelating resin membrane approach which seeks to mimic the action of plant roots (Liang and Schoenau, 1995) and determination of total copper by XRF. Results were correlated with various characteristics of the materials. Parameters measured were total C, N, S, functional groups as determined by FTIR, organic matter content and various characteristics of water extracts of the materials (pH; DOC; NH_4^+

and NO_3^- N; absorbance at 280nm, 465nm, 665nm and phytotoxicity as determined by cress seed germination). Finally, conclusions were drawn as to the maturity of the materials generated; the relative merits of the different approaches used to measure copper; the chemical changes that the copper had undergone during the composting process and relationship between measured copper and various characteristics of the materials.

Chapter One

Literature Review.

1.1 Waste Management in the UK

Waste and its management is an issue of considerable importance within the EC. According to UK government figures (DETR, 2000a), in England and Wales alone around 400 million tonnes of waste are produced every year, the bulk of which goes to landfill. Production of municipal waste is increasing at a rate of 3% each year and as the number of landfill sites is falling this is clearly unsustainable. Also, housed livestock produces 80 million tonnes of waste and the general public generates a further 35 million tonnes in the form of sewage. The EU introduced the landfill directive (EC, 1999) which came into force on 16th July 2001 and set tough targets for the reduction of biodegradeable municipal waste sent to landfill with the ultimate aim of the reduction of landfilled biodegradeable municipal waste to 35% of that produced in 1995 by 2020.

In response to this the UK Government produced *'Waste Strategy 2000'* (DETR, 2000a), outlining the state of waste management in England and Wales at the time and establishing national targets for the recycling/composting of household waste and recovery of municipal waste either by recycling, composting or other forms of material recovery, or recovery of energy. By the end of 2015 at least 33% of household

waste is to be recycled or composted and value should be recovered from 67% of municipal waste.

Unaudited figures recently released by DEFRA (DEFRA, 2005) estimate that recycling of household waste has doubled since 2001 and that English households recycle at levels approaching 23% - more than a fifth of household waste produced. Clearly, much progress has been made.

Strategies for achieving these targets have been stipulated. Broadly speaking, each authority was placed under a statutory requirement to produce its own Municipal Waste Management Strategies (MWM Strategy) in order to double its current recycling and composting rates by 2003-04 and triple it by 2005-06 (DETR, 2001b). Waste reduction is also necessary and is to be achieved on the basis of the Best Practical Environmental Option (BPEO) for that area.

1.2 The Need for Composting

Composting is therefore recognised in the UK at Government level as being an integral part of a policy of sustainable waste management. It also features in the new soil strategy for England (DETR, 2001c) which seeks to develop policy objectives on the management of the extent and diversity of soils and on maintaining and improving the quality of soils. Under ‘Best Value’ composting is classed as waste performance indicator BV 82b and is defined as “...the controlled biological decomposition and

stabilisation of organic substrates, under conditions that are predominantly aerobic and that allow the development of thermophilic temperatures as a result of biologically produced heat. It results in a final product that has been sanitised and stabilised, is high in humic substances and can be used as a soil improver, as an ingredient in growing media, or blended to produce a top soil that will meet British Standard BS 3882, incorporating amendment No 1 (British Standards Institute, 1994). In the case of vermicomposting these thermophilic temperatures can be foregone at the point the worms are introduced." (DETR, 2001b).

Appropriate amendments of composted waste can assist with improving soil quality by improving soil fertility, structure, water retention and workability. They can help in the prevention of soil erosion; land restoration, used as an alternative to peat products; improve the quality of damaged soils and also act as an amendment to soil-forming materials such as quarry wastes.

Soil organic matter is often enhanced in agricultural situations by the applications of manures, slurries and sewage sludge. Advice on the timing and rates of manures and slurries are covered by the Soil Code (MAFF, 1998) to prevent build up of metals, particularly copper and zinc, in the soil. The use of sewage sludge is primarily controlled by The Sludge (Use in Agriculture) Regulations 1989 and its associated amendments (SI, 1989 and 1990), the Code of Practice for the

Agricultural Use of Sewage Sludge (DoE, 1996) as well as the Safe Sludge Matrix (ADAS 2001a and b). Use of untreated sewage to food crops has been banned since from 31st December 1999 and on specified industrial crops for non-food use (e.g. willow and poplar grown for coppicing) after 31st December 2005. After this date only the use of treated sludge will be permitted. These steps are mainly concerned with pathogen control but will also help to avoid the phytotoxic effects seen in soils amended with sewage sludge rich in potentially toxic elements (PTE's – e.g. Cu, Zn, Ni). Some, like zinc and copper, are essential to plants and animals in trace amounts but can be toxic at higher levels and have been seen to be taken up in greater quantities from sludge-treated soils compared with non-treated soils (Davies and Jones 1988).

Composting is a simple and useful way of treating such wastes because they are organic and can therefore be easily returned to the soil. However, there can be problems if the raw materials are contaminated with heavy metals and/or organic compounds and these will be magnified due to the concentrating effect of the composting process. Although much reduced in recent years heavy metals continue to be common constituents of industrial waste streams, arising from activities such as tannery and metal-working processes. On 26th January 2004 new EC regulations governing the levels at which trace elements could be used to supplement feedstuffs came into force (EC, 2003). From this date the maximum level at which adult pigs could receive copper as a supplement was reduced to 25 mg copper per kg of feed. Before this time pigs

between 4 and 6 months of age were routinely fed up to 100mgkg^{-1} under an earlier directive (EC, 1984), 95% of which was excreted in the pig's faecal matter producing slurries high in copper. Most heavy metals when present in soil at low concentrations act as plant micronutrients and take part in important biochemical pathways. Composts produced from metal-rich slurries can therefore have beneficial effects when applied to soils suffering from deficiencies. However, at high concentrations they are usually toxic and give rise to stunted growth and chlorosis. There are some exceptions to this. Members of the Cruciferae family (eg. broccoli) hyperaccumulate zinc in order to render them inedible to predators. Metals are also known to be toxic to soil microorganisms, indeed copper has been demonstrated to eliminate nitrogen fixing bacteria in low concentrations (Keeling and Cater, 1998)

Therefore, for the end product to be acceptable for use in defined circumstances the compost must meet standard criteria such as British Standards Institution Publicly Available Specification for composted material or BSI PAS 100:2005 (WRAP, 2005), developed by the British Standards Institute in conjunction with the Waste Resource Action Programme and The Composting Association (TCA). Composting of sewage sludge cake is permissible according to this standard. However the limit for copper is 200 mgkg^{-1} (WRAP, 2005).

Investigating the forms in which copper exists in wastes and the ways in which it changes is of vital importance to understanding the mechanisms of release into the environment and subsequent bioavailability.

1.3 Metal Speciation in the Environment

Historically heavy metal content has focussed on total analysis i.e. an estimation of the total pool. Such an approach means that all forms of a given metal are implicitly considered to have an equal impact on the environment (Martin *et. al.* 1987). This approach was gradually abandoned as discrepancies between total content and availability arose (Viets, 1962). Athough legislation is still based on total metal contents (MAFF, 1998), it is now generally recognised that total contents yield little useful information about bioavailability.

Bioavailability, toxicity and transport of heavy metals within the environment is determined by their specific chemical forms (Chwastowska and Skalmowski, 1997; Quevauviller *et. al.,* 1997) or the types of binding in which they take part (Quevauviller *et. al.,* 1997). Parameters affecting bioavailability include soil solution pH, organic matter content, cation exchange capacity (CEC), redox potential (Eh), microbial population and, most importantly, whether or not metals have been incorporated into the soil as inorganic salts or in forms bonded to organic matter (Ciavatta *et. al.,* 1993). Assessment of the uptake of cadmium and lead from sludge-amended soil by perennial ryegrass

(Hooda and Alloway, 1993) is one example. Here bioavailability was seen to be influenced by sludge application rate, temperatures, pH and whether or not the soils had been artificially contaminated with nitrate salts of the metals.

Viets (1962) developed the concept of chemical 'pools' of micronutrient cations within soils based on exchange and solubility reactions; each element in each pool differing in concentration, size, turnover rate and equilibrium with other pools of that element. Five hypothetical pools were described in detail and in relation to each other. Represented diagrammatically (Fig. 1.1) they were to be used as a useful model to qualitatively assess micronutrient solubility and availability.

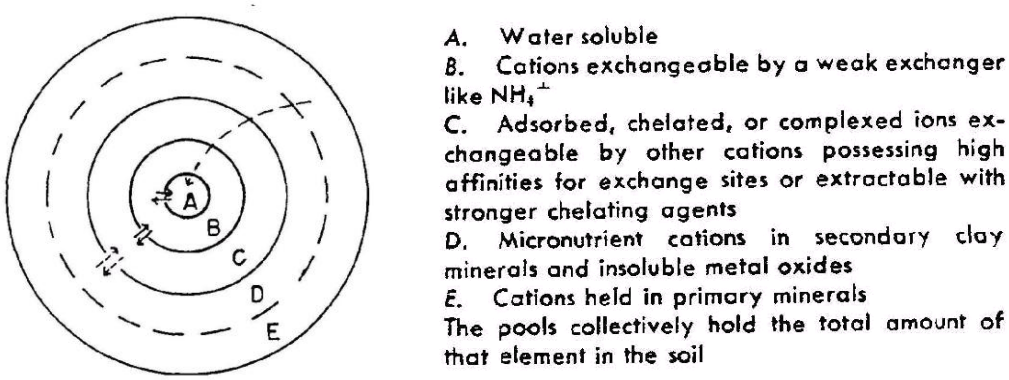


Fig. 1.1 The hypothetical pools of micronutrient cations (Source: Viets, 1962).

The role of organic matter at this stage was unclear. What seemed to be apparent was that behaviour depended on the micronutrient involved, pH, Eh, soil type and the type of organic matter involved. Humus did appear to be "active in complexing or chelating micronutrients and may be the absorbent of greatest importance for pool C in many soils" (Viets, 1962).

Indeed, such is the importance of humus as a chelating/complexing source and sink for micronutrient cations in soils that cation extraction is now performed as standard by ADAS (MAFF, 1986) using a solution of the strong chelating agent EDTA. Since then these ‘pools’ have been identified as including exchangeable amorphous oxides of Fe, Mn and Al; organic matter; carbonates; phosphates and sulphides and have continued to be recognized as playing a fundamental role in environmental heavy metal chemistry and bioavailability.

Analysis of metals within these hypothetical pools is often now referred to as ‘speciation’, a term which has caused a great deal of controversy. It is often referred to in two different ways. The first approach refers to the determination of well-defined chemical species, or the different oxidation states of an element. Strictly speaking this is the correct use of the term ‘speciation‘.

The second approach is used with reference to fractions of an element in a material which have been determined after a separation process. This is a measurement of association forms rather than discrete compounds and is really a misuse of the term speciation as the elements are dissolved and their specific bonds to the solid phase lost. The emphasis is on the concept of subdividing a “total content” (Tack and Verloo, 1995). In the past, metals detected in this manner were often related to specific fractions of the solid phase e.g. ‘carbonate bound’ rather than ‘acid soluble’ and extraction methods were credited with too much specificity.

Metals detected in this way really should be classified as belonging to ‘operationally defined’ fractions (Rauret and Rubio, 1997), i.e. the process employed defines the fraction obtained (Perez-Cid *et. al.,* 1996). Both approaches are very different from each other but the term ‘speciation’ has often been applied to both indiscriminately.

Ure (1991) went further. He stated that speciation is:

1. The *active process* of identification and quantification of the different *defined species, forms or phases* in which an element occurs in a material.

2. The *description* of the amounts and kinds of the species, forms or phases. For soils these can be specified in three different ways:

a. *functionally,* i.e. by their role e.g. plant-available

b. *operationally*, by the reagents or procedures used to isolate and identify them.

c. *specific chemical compounds or oxidation states* of an element e.g. ferrous iron.

Speciation in terms of defining specific chemical species is extremely difficult because of the numerous reactions taking place on various surfaces at any one time. Charlet and Manceau (1992) believed that all these surface processes should be described in detail so that possible pathways of detoxification of the heavy metals, when present at hazardous concentrations could be understood.

1.4 Operationally Defined Speciation

Defining specific chemical species within environmental matrices is extremely difficult and usually dependant upon expensive, high specification instrumentation. In many cases it may not even be possible. The subject of an instrumental approach towards the speciation of metals in soils has recently been very thoroughly reviewed (D'Amore *et. al.,* 2005) and is not the subject of discussion here. Needless to say the methods outlined are particularly difficult to apply in natural systems rich in organic molecules undergoing continuous change, with many different binding sites competing with each other and with dissolved ligands for the metal ions.

The alternative therefore is to take an 'operational approach'. Within solid matrices (soils, sediments and composts) this has been the subject of discussion in a number of reviews (e.g. Tack and Verloo, 1995). It generally employs extraction techniques and subsequent detection of the metal using for example AAS or ICP-OES. The extraction step tries to separate as cleanly as possible the metal from specific phases within the biological material. The determination of these 'operationally' or 'functionally' defined phases performs three functions:

i) Assesses likely phytoavailability (Quevauviller *et. al.,* 1997);

ii) Reveals distributions patterns within soil profiles and

iii) Assesses the storage efficiency of the soil as a medium for storing metals and explains ways in which metals might be leached (Keller and Vedy, 1994).

Early work (e.g. Viets, 1962) concentrated on the use of single extractants applied to soils and sediments but in recent years soil amendments e.g. composts and sewage sludges have begun to receive more attention. As with soils and sediments it is recognized that the 'determination of total amounts of elements in compost is insufficient for a correct evaluation of a compost's quality. It is necessary to carry out a speciation analysis' (Chwastowska and Skalmowski, 1997). A short term lab-based composting experiment (Ciba *et. al.,* 1997) demonstrated this well. Metallic zinc applied to composting materials was observed to remain in mobile and bioavailable forms whereas 70% of applied zinc sulphide remained in unavailable forms.

Diagnostic tests for heavy metal phytoavailability in soils have also often concentrated on the use of single extractants of the metal. Amounts extracted are then correlated with their uptake by plants grown under optimal conditions, effectively providing a functional assessment of speciation. Popular extractants include water (Petruzelli, 1985), EDTA (MAFF, 1986), DTPA (Handreck, 1994) and NH_4OAc (MAFF, 1986) as these have been shown to correlate well with plant uptake.

Martin *et. al.* (1987), after reviewing the literature, stated that use of one-step extractions has been found to be advantageous in that they:

i) Are rapid and easy to perform

ii) Show clearly differences between abnormal and background levels.

iii) Can be used as a first 'look-see' approach before applying sequential extraction procedures (Chester *et. al.,* 1985).

But that they do have disadvantages, namely:

i) Results depend upon the type of sample used (Chester and Hughes, 1967).

ii) Readsorption can occur at neutral pH (Malo, 1977; Rendell *et. al.,* 1980)

iii) Do not permit the dissolution of all the organic and inorganic labile forms without also attacking the detrital ones.

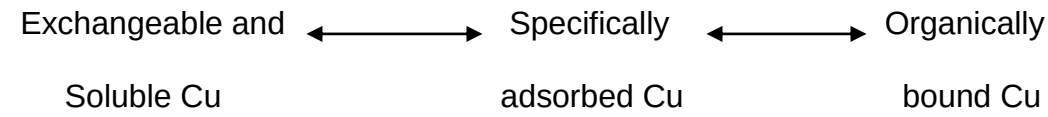
Usefulness of extractants such as DTPA and NH_4OAc in predicting phytoavailability of Cu and Zn in soilless potting media decreases when the media is heavily contaminated with sludge-Cu and Zn (Handreck, 1994) and the same has been observed for Cd and Pb uptake by perennial ryegrass from soil mixed with sewage sludge (Hooda and Alloway, 1993). Also, it must be borne in mind that single extractants are generally not phase specific and sometimes the phase itself is often ill-

defined. Therefore, as previously discussed, the metals extracted are often best described operationally by the procedure or reagent itself.

1.5 Copper Retention by Organic Matter

Within mature composts, copper speciation is essentially concerned with the formation of organic complexes and compounds i.e. retention by the fraction that would be defined as pool C in soils and sediments according to Viets (1962). It is therefore worth reviewing the ways in which copper is known to combine with organic compounds.

Heavy metals are commonly defined as being those metallic elements with a density > 5000 kg m^{-3} (McGraw-Hill, 2003). Copper, having a density of 8930 kg m^{-3} at 20^{o}C belongs to this class (Nayer, 1997) and since 1931 has been recognised as being essential to plant growth (Lipman and Mackinney 1931; Sommer 1931) though only small amounts are required. Deficiencies are common, particularly in peaty or organic soils as a result of the formation of highly stable complexes with the COOH (carboxylic) functional groups on the surfaces of the organic material, and it is these complexation processes, in particular, which control copper bioavailability. It has been concluded that phytoavailable copper from soils is controlled by the equilibrium between exchangeable/soluble copper, specifically adsorbed copper and equilibrium with organically bound copper (McClaren and Crawford 1973):



Exchangeable and soluble copper

With regard to phytoavailability, soluble copper is the most important form which needs to be considered. Copper (like other metals) does not exist in water simply as Cu^{2+} but as a hydrated complex (Stevenson, 1994) with the oxygen end of the water dipole oriented towards the positively charged cation Cu^{2+}. Most of the copper found in soil solutions (particularly peats) has been seen to be organically complexed (Stevenson, 1994). Here one or more water molecules surrounding the metal cation are replaced by other molecules or ions. An organic molecule that combines with the cation in this way is known as a ligand and the bonding involved is mainly, though not always, covalent (electron pairs are shared between two atoms) with coordinate linkages being formed. These bonds arise because the outer electron shell of the copper cation is not completely filled and is able to accept unshaired pairs of electrons from functional groups that possess them. These groups vary in their ability to coordinate with metals, some having a greater affinity for them than others. Binding abilities are generally of the order carbonyl (C=O) < ether (-O-) < carboxylate (-COO-) < ring N (-N=) < azo (-N=N-) < amine ($-NH_2$) and enolate (dissociated phenolic –OH, O^-) (Cresser *et. al.,*1993). Other types of bonding occur but these are discussed later in the text.

Cu^{2+} is unusual in that it will form complexes with ligands containing oxygen as a donor atom (i.e. a Class A metal ion) as well as ligands containing N,P and S donor atoms (i.e. a Class B metal ion) (Stevenson 1994). Not only that but it forms the strongest of all chelate complexes. The term chelate comes from the Greek *chele* meaning crab's claw and such complexes arise where two or more coordinate positions are occupied by donor groups from a single ligand acting in a pincer-like fashion. A ring-like structure is formed and thus the metal is bound very strongly. An example of the formation of two such complexes is given in Fig 1.2.

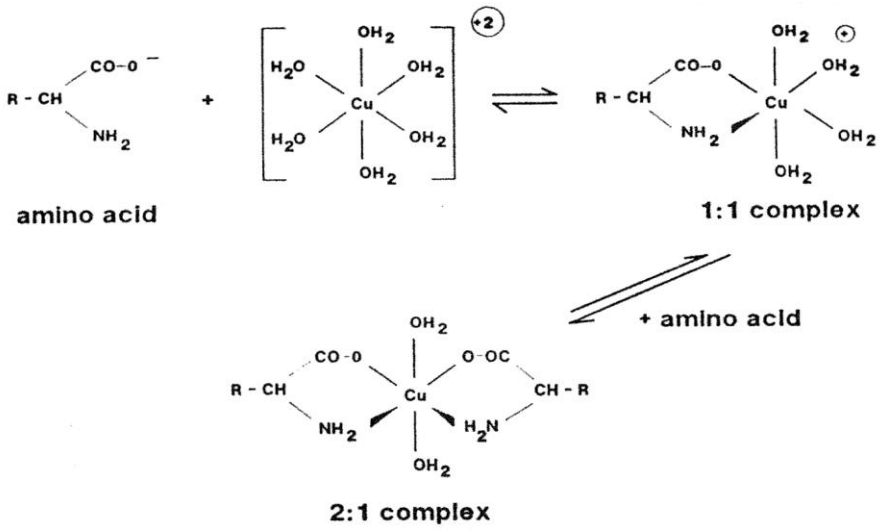


Fig 1.2 Formation of 1:1 (bidentate) and 2:1 (*bis* bidentate) Cu^{2+} complexes with an amino acid (Source: Stevenson, 1994)

<u>Specifically Adsorbed / Organically Bound Copper</u>

Organic matter has a large cation binding capacity as a result of its numerous highly reactive oxygen containing binding sites from both humic and fulvic acids. Both basic functional groups (e.g., -NH_2 amino, =O carbonyl, -OH alcohol and –S- thioether) and acidic ones (e.g., -COOH carboxyl, -OH enolic or phenolic and –SH thiol) play their part and coordinate with the hydrated copper ions (Tejowulan, 1999). The complexity of organic materials is such that a large assortment of reactions is believed to take place ranging from the involvement of weak ionic forces (Van der Waals forces) to the formation of very strongly bound chelate structures. Such reactions are much the same as the outer and inner sphere complexes formed at the cation-mineral interface

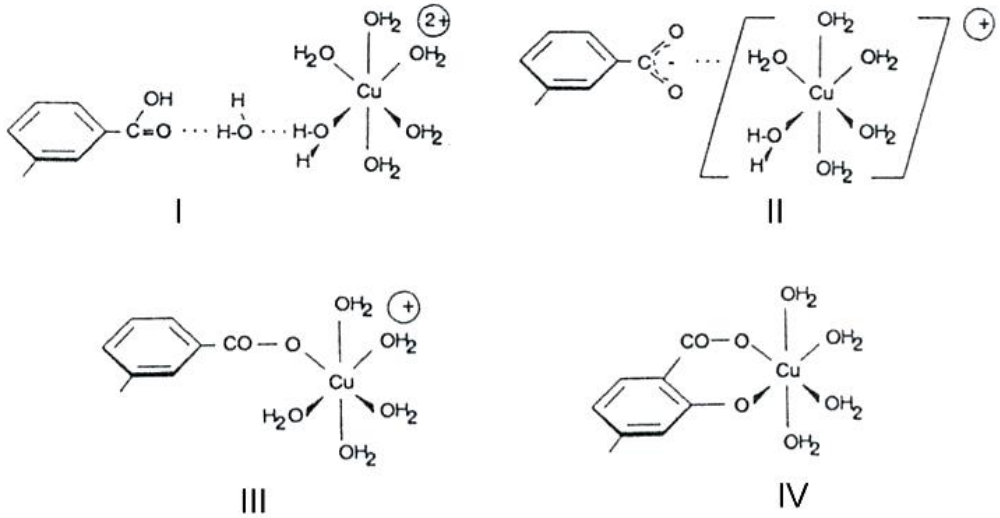


Fig. 1.3 Binding of Cu^{2+} by various functional groups from humic substances I = water bridge; II = electrostatic attraction to a charged COO^- group; III = formation of a coordinate linkage with a single donor group and IV = formation of a chelate structure, such as with a COO^- - phenolic OH site combination (Source: Stevenson 1994).

in soils. Stevenson (1994) outlines a number of examples which are believed to occur (Fig. 1.3). Binding occurs first at sites which form the strongest complexes (examples III and IV in Fig 1.3) and only when these sites become saturated do sites to which metals are less strongly attracted (i.e. I and II in Fig 1.3) become important.

Complex Characteristics

The tendency of a ligand to form a complex with any cation is defined in terms of the formation or stability constant of the reaction. The reaction is described by the following equation:

$$nM + mL \longleftrightarrow M_nL_m$$

with the stability or formation constant, K, being

$$K = \frac{[M_n L_m]}{[M]^n [L]^m}$$

Here [M] is the trace element concentration, [L] is the ligand concentration and [ML] is the concentration of the metal-ligand complex. Subscripts and superscripts n and m are the number of moles of metal ion and ligand molecules taking part (Stevenson, 1994). Thus, in Fig.

1.3., example IV will have a greater value for K than example I. The strongest complexes of all are multidentate i.e. the metal chelates with two or even more organic functional groups.

As a general rule the most strongly bound metals are the most slowly desorbed because larger activation energies are required to break the stronger bonds. Dissociation rates of adsorbed metals from organic surfaces are several orders of magnitude slower than their adsorption (Tejowulan, 1999) rates. So slow are they that it is generally believed that many of these processes are irreversible, especially at higher pH values where multidentate complexes dominate. The abilities of the phenolic (OH) and carboxylic acid (COOH) groups to bind with or dissociate from metal ions is strongly dependent on several factors, namely:

1. pH
2. Number and type of functional groups,
3. Electrolyte composition of the water fraction (and hence ionic strength) within the material.
4. Degree of saturation of the binding sites.
5. Structure of the organic matter

Changes in pH alter the extent to which the COOH groups dissociate and also effects hydrolysis of metal ions with increases in pH leading to increases in hydrolysis. In water, COOH groups of humic and fulvic acids dissociate to form COO^- and H^+ and the organic molecule then stretches

out due to repulsion of the negative charges (Fig. 1.4). When metal ions are then introduced into the system, humic and fulvic salts form and the

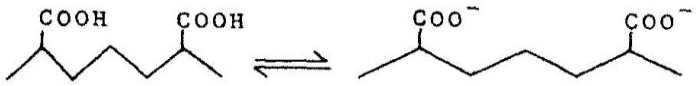


Fig. 1.4 Ionisation of COOH groups and subsequent

stretching of the organic polymer (Source: Stevenson 1994).

negative charges become satisfied. The organic molecule then collapses on itself and may become insoluble. Polyvalent cations such as Cu^{2+} may also link individual molecules together to produce long chain-like structures which, as they grow are apt to precipitate (Stevenson, 1994). Work carried out by Lead *et. al*. (1998) found that increases in pH lead to a corresponding decrease in metal binding to suspended particulates matter taken from the River Mersey. This was felt to be a reflection of the effect of competition by protons for surface binding sites and perhaps to a lesser extent be linked to the larger negative surface charges at high pH.

Electrolyte composition is also influential on Cu^{2+} uptake by humic substances with increases in ionic strength promoting ionisation of the functional groups. The complexation capacity of a humic acid has been found to drastically reduce with increases in salinity (Carter, 1992). Coupled with this effect, additional salts compete with the copper ions for

organic ligands. The activity coefficients of the metal ion are also strongly affected by ionic strength.

Arp (1983) found that the physical structure of the organic polymer itself is important. It was noted that the occurrence of charges on the molecule lead to electrostatic attraction or repulsion. Inter and intra-molecular H-bonding and burial of acidic groups was also discovered.

All these parameters change as materials compost and breakdown. It therefore follows that Cu^{2+} complexation patterns and hence phytoavailability during the composting process must also change.

1.6 Sequential Extraction Schemes – An Introduction

It has been in established in section 1.2 that the speciation of metals in complex, constantly changing, organic materials, such as composts, must currently be done using operational approaches.

Since the 1960's sequential extraction procedures have been developed to improve specificity by aiming to limit the access of an extracting reagent to no more than a single specific phase. The principle behind them is based on Viet's concept of pools and involves the use, in sequence (i.e. 1,2,3,4... *etc.* steps), of reagents of increasing strength, aiming to remove increasingly immobile metals from the main components (Bermond and Benzineb, 1991). The sequence in which the

extractants are used has been found to be important (Miller, Martins and Zelazny, 1986).

Originally designed for the analysis of sediments their usage has widened considerably and they have proven popular in the study of changing environments such as estuaries and recently deposited sediments (Martin *et. al.,* 1987). They have shown themselves to be a pragmatic analytical tool (Tessier and Campbell, 1991) and it is generally agreed that they provide a more precise definition of the forms in which metals exist and a knowledge of the mechanisms involved in the release of elements to the environment under natural conditions (Chwastowska and Skalmowski, 1997).

Schemes are many and varied but the one most often used is that developed by Tessier and co-workers (see Table 1.1) although it is often applied in a modified form, usually with changes made to stage 5 to accommodate those laboratories unable to use HF.

It is believed that metals in the exchangeable fraction are likely to be affected by changes in water ionic composition as well as sorption-desorption processes and so are readily available both for plants and for leaching. Metals in the so-called carbonate fraction, present in mineral soils and sediments, are susceptible to changes in pH and the oxide-bound or reducible fraction which is thermodynamically unstable under anoxic (oxidising) conditions are considered to be relatively labile and

may be potentially bioavailable to the environment. Metals in the residual fraction though are regarded as being tightly bound in mainly primary and

Table 1.1

Tessier Sequential Extraction Scheme (Source: Tessier *et. al.,* 1979)

Stage	**Fraction**	**Reagent**	**Shaking time and temperature**
1	Exchangeable	8 ml of $MgCl_2$ 1mol dm^{-3} (pH = 7)	1hr at 25^{o}C
2	Carbonate-bound	25 ml of NaOAc 1 mol dm^{-3} (pH = 5)	5 h at 25^{o}C
3	Fe-Mn oxides	20 ml $NH_2OH.HCl$ 0.04 mol dm^{-3} in HOAc 25% m/m	6 h at 96^{o}C
4	Organic matter	3 HNO_3 0.02 mol dm^{-3} + 5 ml H_2O_2 30% m/v 3 ml H_2O_2 30% m/v 5 ml NH_4OAc 3.2 mol dm^{-3} in 20% HNO_3	2 h at 85^{o}C 3 h at 85^{o}C 30 min at 25^{o}C
5	Residual	1 ml HCl 35% m/m + 2 ml HF 48% m/m+ 4 ml HNO_3 70% m/m + 5 ml H_2O	Digestion procedure for total metal analysis.

secondary minerals. These would not be expected to be released into solution over a reasonable time span under the conditions normally encountered in nature (Zorpas *et. al.,* 2000, Hsu and Lo, 1999 and Ciba *et. al.,* 1999). Thus, assessment of the bioavailability of contaminants and ameliorative measures for polluted sediment are two examples where sequential extractions may provide useful information (Whalley and Grant, 1994).

Though developed for use in soils and sediment analysis these schemes have also been applied, unchanged, to composts. Examples and a discussion of these are given later on in section 1.8 but first it is important to consider both the advantages and disadvantages inherent in the use of sequential extraction schemes.

1.6 Sequential Extraction Procedures - Their Problems

Despite their popularity they are not without their limitations. Their accuracy and legitimacy is sometimes called into question and heated debates have arisen (Nirel and Morel, 1990; Tessier and Campbell, 1991). Objections raised concern work performed on soils and sediments but are just as applicable to other matrices. Most of the issues raised centre around the following objections and are dealt with in detail:

i) A lack of specificity/selectivity of the reagents
ii) Readsorption of the analytes on to mineral surfaces during extraction or precipitation.
iii) Lack of standardised methods.
iv) Procedures are complicated, time-consuming and prone to errors

Lack of specificity/selectivity of the reagents

The use of sequential extraction schemes tends to assume the extraction or dissolution of discrete phases i.e. they are meant to be 'phase selective' (Miller *et. al.,* 1986a). This is probably not the case. None of the elements extracted from model aquatic sediments (Kheboian and Bauer, 1987) were extracted at the expected stage of the proceedings; sequential analysis of suspended river particulates destroyed cellular matter during the first extraction step (Lead *et. al.,*1998); and failure of

carbonates to dissolve during step 2 but then subsequently dissolve during step 3 has also been seen to lead to an overestimation of the 'iron and manganese bound fraction' (Tack and Verloo, 1996). Other problems encountered include analysis of forest soils (Keller and Vedy, 1994), clean and contaminated synthetic soils and a naturally occurring silt loam (Kim and Fergusson, 1991) and humic acids mixed with non-doped mineral phases (Xiao-Quan and Bin, 1993).

Readsorption of analytes on to mineral surfaces during extraction or precipitation

Readsorption during extraction has been reported by a number of workers and is where metal initially released by a reagent re-precipitates or partitions back onto the solid phase. This has been seen in the study of model sediments (Kheboian and Bauer, 1987); a model soil (Xiao-Quan and Bin, 1993); copper precipitation in a range of sediments (Barbanti and Sighinolfi, 1988). Certainly there is also a perceived danger that dissolution of solid phases at each step will lead to a shift in the equilibrium between the solution and remaining phases, though the extent to which this occurs is unknown (D'Amore *et. al.* 2005).

Lack of standardised methods

Many different schemes have been used although they are mainly based on Tessier's scheme (Tessier *et. al.,* 1979) or a modification of it

(Chwastowska and Skalmowski, 1997). However, it has been claimed that there have been so many proposed that results between laboratories cannot really be compared (Rauret and Rubio, 1997).

In an attempt at harmonisation, the Community Bureau of Reference (BCR) [now known as the Standard Measurements and Testing Program (SMT)], of the Commission of the European Communities eventually recommended a three stage SE procedure for sediments and a single extraction method for the speciation of heavy metals in soils (Ure *et. al.,* 1993), the aim being to strike a compromise between analysis time and information obtained (Perez-Cid *et. al.,* 1996). Certified reference materials for use with these procedures were also prepared (Quevauviller *et. al.,* 1997). Fewer matrix effects from the reagents were seen with the BCR scheme compared with the Tessier scheme (Perez-Cid *et. al.,* 1996); within sample replication was good and fraction 1 of the BCR scheme was approximately equal to fractions 1 and 2 of the Tessier scheme. For both procedures copper was mainly associated with the so-called ‘organically bound’ fraction or ‘oxidisable’ fraction.

The BCR scheme is now considered by many to be a standard method (Filgueiras *et. al.,* 2002). However, it is the opinion and experience of this author that as the considerable amount of effort and time required to carry out this procedure is equal to that of the Tessier scheme and only yields three extracts then it is far better to use Tessier’s approach which does at least yield five. However, it may well be necessary under certain

circumstances to adopt this ‘standard’ technique if the aim of the analysis is to be able to compare results with other workers who also use this particular SE scheme.

Procedures are complicated, time-consuming and prone to errors

SE procedures are indeed time-consuming with a batch of samples taking anywhere between 3-5 days just to generate just the extracts although attempts at speeding up the process using microwaves have been reported (Dutta *et. al.,* 2005; Dutta and Das, 2005) and with some success. Analysis time depends entirely on the number of samples to be analysed, technical skill of the operator and availability of appropriate equipment. In most cases it cannot be considered to be a routine analytical approach (Chwastowska and Skalmowski, 1997). Further difficulties may also be experienced when amounts of the metal/s being analysed are of the order of μgkg^{-1} (Keller and Vedy, 1994).

Other limitations of the SE approach should be considered:

1. Nature and treatment of materials and extractants – the outcome is heavily influenced by the duration, effect of grinding, temperature and the ratio of the amount of solid matter to extractant volume (Martin *et. al.,* 1987; Potter *et. al.,* 1991), the most severe errors occuring at low substrate to extractant ratios (Rauret *et.al.,* 1999).

2. Variable Recoveries - this has been described in detail by Kheboian and Bauer (1987) and attributed to a number of factors including loss of metals during the intermediate water washes and violent reactions during step 4 leading to sample losses. However, the chief source of error was deemed to be the large number of manual manipulations involved. Recovery of copper from a humic acid (Whalley and Grant, 1994) during sequential extraction using the BCR scheme varied between 19 and 61%. Metal release was also found to vary between repeated experiments although patterns of distribution remained the same. Kim and Fergusson (1991) also found variable recoveries although within sample variation was low. Within the context of compost analysis, such variable recoveries may result in the failure to detect changes which occur during the composting process.
3. Fails to mimic natural processes – this was one of Nirel and Morel's (1990) chief objections. They argued that SE schemes employ "strong reagents and fast kinetics" whilst natural conditions involve "weak reagents, slow kinetics". Other workers also feel that they do not allow predictions to be made about the intensity and kinetics of metal release in the field (Tack *et. al.,* 1999).

Few authors (D'Amore *et. al.,* 2005 are the exceptions) make the most obvious objection, which is that SE procedures were originally developed for use on sediments where the analytes of interest are at trace metal levels and not on general solid samples where analytes are actually

major constituents and often of anthropogenic origin and have not occurred naturally.

Despite their obvious drawbacks though the technique remains popular and is still felt to play a useful role in the operational speciation analysis of environmental samples. The advantages such an approach brings are therefore worthy of consideration.

1.8 Sequential Extraction Procedures – In Their Defence

Sequential extraction schemes have come in for a barrage of criticism (Nirel and Morel, 1990). Tessier and Campbell (1991), in defence of the technique made two points:

i) Assessment of metal readsorption during the extraction process has largely been carried out using artifical sediments composed of phases prepared in the laboratory.
ii) Operational does not equal uselessness.

Assessment of metal readsorption during the extraction process has largely been carried out using artifical sediments composed of phases prepared in the laboratory.

The use of artificial systems, particularly where spiked phases are mixed together has serious flaws in that these phases are not representative of

anything found in natural systems. These unnatural, phases are bound to result in the redistribution of the metals. Tessier and Campbell did not rule out the use of model systems but they pointed out that extrapolation of the results to natural systems is ‘tenuous at best’. They suggested that spiking natural systems might be a more realistic as well as a more straightforward approach.

Operational does not equal uselessness.

Tessier and Campbell asserted that operational assessments have proved to be invaluble in the ‘description and prediction of biological trace metal uptake by sediment-dwelling organisms and aquatic plants’ and believed the sequential extraction analyses are a ‘pragmatic analytical tool ...provided that they are used with discrimination and care’, useful for dealing with practical problems e.g. fertilizer recommendations and setting of legislation governing the limits of application of useful yet potentially hazardous soil amendments, often when more detailed information about soil processes have been lacking.

It is true that many workers within the scientific community have been rightly accused of over-interpreting their results, assigning mineral phases where there are none. Even now this mistake persists (e.g. Gao *et. al.,* 2005) but they are the ones who are at fault, not the procedure.

Kersten and Forstner (1986) go further and suggest that the operational species be defined in terms of the extractant used rather than the phase or mode of retention assumed to be attacked during the extraction process.

As long as they are used with care and their limitations are borne in mind there is no reason why sequential extraction procedures cannot continue to be very useful. Others have also spoken in their defence (Barbanti and Sighinolfi, 1989) and even the somewhat sceptical view taken by D'Amore *et. al.* (2005) acknowledges that they can be a 'useful tool in metal partitioning' although they then qualify their statement by saying they should always be confirmed by other methods.

1.9 Sequential Extraction Procedures and Compost (With Particular Emphasis on Copper)

Examples in the literature of SE schemes being applied to whole, composted or composting materials (or size separated fractions of composts) are somewhat lacking in the literature. To date only some forty or so examples exist. Those found are listed in Table 1.8.1.

The composts used vary from those produced at large composting plants (e.g. Chwastowska and Skalmowski, 1997) to those created in the laboratory (e.g. Zorpas *et. al.,* 2000) and time taken to produce them has ranged from a mere, fifty hours (Ciba *et. al.* 1997) - in which case the

Table 1.2

Sequential Extraction Schemes Applied to Composted Materials.

Author	Metals of Interest	Compost Substrates*	SE* Scheme	Notes
Huang *et. al.* 2005	Cu + Zn	Pig manure and sawdust	Tessier *et. al.* 1979	Changes over time investigated
Gao *et. al*. 2005	Cu, Zn + Cd	SS	Tessier *et. al.* 1979	Changes over time investigated
Szymanski *et. al.* 2005	Cu, Pb + Zn	MSW	Own, derived from Tessier *et. al.* 1979; Lo and Yang 1998 and Scancar *et. al.* 2001.	Changes over time investigated
Bhattacharyya *et. al.* 2005	Cr	MSW	Carbonell-Barachina *et. al.* 1999	Analysis of end products only
Amir *et. al.* 2005	Cu, Zn, Pb + Ni	SS	Sposito	Changes over time investigated
Zheng *et. al*. 2004	Pb	SS	Tessier *et. al.* 1979	Changes over time.
Liu *et. al.* 2003	Cu, Zn, Fe + Mn	Products from seafood processing, MSW, pig manure + SS	Modified Tessier *et. al.* 1979	Analysis of end products only
Ciba *et. al.* 2003	Fe, Cu, Zn, Cr, Ni, Pb, Cd + Hg	MSW	Tessier *et. al.* 1979	Analysis of end products only
Bhattacharyya *et. al.* 2003	As	MSW	Carbonell-Barachina *et. al.* 1999	Analysis of end products only. Bioavailability assessed.
Zorpas *et. al.* 2003	Metals	Dewatered, anaerobically stabilized primary sewage sludge.	Modified Tessier *et. al.* 1979	Starting and end products only
Greenway + Song 2002	Metals	Mixed wastes	BCR + ultrasound	Analysis of end products only
Greenway + Song 2002	Metals	Various green wastes	Modified BCR	Changes over time investigated
Hsu + Lo 2001	Cu, Mn + Zn	Separated swine manure	He *et. al.* 1995	Changes over time investigated
Korolewicz *et. al.* 2001	Zn, Cd, Cu, Ni + Pb	Organic fraction of MSW containing cellucotton	Rudd 1988	Starting and end products only
Chwastowska + Sterlinska 2000	Cd, Pb, Cu, Ni, Co, Cr + Zn	MSW	Modified BCR	Analysis of end products only
Zorpas *et. al.* 2000	Cd, Cr, Cu, Fe, Mn, Ni, Pb + Zn	SS + clinoptilolite	Modified Tessier *et. al.* 1979	Starting and end products only
Egreja *et.al.* 1999	Cu, Pb, Mn + Zn	MSW	?	SE performed on physically separated size fractions of the end product
Planquart *et. al.* 1999	Zn, Cu + Pb	Anaerobically digested sewage sludge + bark and green waste	Modified Tessier *et. al.* 1979	Analysis of end products only. Bioavailability also assessed.
Hsu + Lo 1999	Cu, Mn + Zn	Separated pig manure	Tessier *et. al.* 1979	Changes over time investigated

*Where SE = Sequential Extraction, MSW = Municipal solid waste and SS = Sewage sludge

Author	Metals of Interest	Compost Substrates*	SE* Scheme	Notes
Ciba *et. al.* 1999	Cd, Co, Cu, Mn, Ni, Pb + Zn	Dry MSW and activated sludge	Rudd 1998	Analysis of end products only
Pare *et. al.* 1999	Cu, Zn, Cr, Pb, Ni + Co	Co-composted biosolids, woodchips and MSW	Mathur and Levesque 1983	Changes over time investigated
Ho + Qiao 1998	Cd, Cr, Cu, Ni, Pb, Zn – only Cr reported	Grass clippings + sawdust (MSW) amended with bauxite	Tessier *et. al.* 1979	Changes over time investigated
Chwastowska + Skalmowski 1997	Heavy metals	MSW	Modified Tessier *et. al.* 1979; Tessier *et. al.* 1979; BCR (single extractions), EPA TCLP + Polish leaching test.	Analysis of end products only
Qiao + Ho 1997	Cr, Cu, Ni, Pb + Zn	MSW with and without bauxite	Tessier *et. al.* 1979	Changes over time investigated
Ciba *et. al.* 1997	Zn	MSW	Rudd '88	Changes over time investigated
Chwastowska *et. al.* 1996	Heavy Metals	MSW	Tessier *et. al.* 1979	Analysis of end products only
Tisdell + Breslin 1995	Cd, Pb, Fe, Cu, Cr, Ni + Zn	MSW	Fraser + Lum 1983 (Tessier *et. al.* 1979)	Analysis of end products only
He *et. al.* 1995	Pb, Cd, Mn, Cu, Zn, Cr + Ni	MSW; MSW-SS; food waste + MSW feedstock (shredded and screened, not composted)	Their own	Analysis of end products only
Handreck 1995	Mn	Composted bark, composted rice hulls, peat + eucalyptus sawdust	Warden + Reisenauer 1991	End products with and without Mn amendments.
Bourque *et. al.* 1994	Heavy metals	MSW (poorly separated)		End product
Sterlinska+ Golebiewska 1994	Cr	MSW	Tessier *et. al.* 1979	Analysis of end products only
Prudent *et. al.* 1993	Cu, Cd + Pb	MSW	Own, derived from Tessier *et. al.* 1979, Dudka + Chlopecka, 1990 and Del Fava 1992.	'Fresh and mature' products only.
Canarutto *et. al.* 1991	Cu, Cd, Zn, Ni, Pb + Cr	MSW	Petruzzelli 1985	0 and 60 days products.
Petruzelli *et. al.* 1989	Heavy metals	MSW	Own	End product

*Where SE = Sequential Extraction, MSW = Municipal solid waste and SS = Sewage sludge .

material cannot really be described as compost - to six months (Planquart *et. al.,* 1999; Canarutto *et. al.,* 1991). Most of them have used municipal solid waste and/or sewage sludge as substrates although occasionally pig manure has also been used (e.g. Hsu and Lo, 2001; Huang *et. al.,* 2005). Reports of composts produced from less frequently used. substrates, such as the by-products of seafood processing (Liu *et. al.,* 2003), cow dung manure (Bhattacharyya *et. al.,* 2005), grass clippings and sawdust (Ho and Qiao, 1998) and, more obscurely, bark, rice hulls, peat and eucalyptus sawdust (Handreck, 1995) are also to be found.

For the most part, sequential extraction analyses are only performed on the end product – the mature compost although some workers have used it to track the changes in metal operational speciation as these wastes breakdown and decompose. Both approaches are entirely valid and the decision to use either depends entirely on the aims of the analyst.

Most researchers employ the Tessier scheme (or a modified version of it) although others have been used (He *et. al.,*1995). Work by Hsu and Lo (2001) on separated swine manure confirmed that, in all cases, copper was associated strongly with the so-called ‘organically bound’ or ‘oxidisable’ fraction of the scheme. They also discovered that water soluble copper increased during the first 18 days of composting and that these increases corresponded with increases in soluble carbon and decreases in copper found in the organically bound and organically complexed fractions. As already explained copper readily forms soluble organic complexes (section 1.4) and this work serves to highlight the

potential dangers of copper release and likely bioavailability when applying raw materials or a compost to land. This would explain why increases in phytotoxicity have been seen when raw sludge is applied to land (Davies and Jones, 1988). The 'reducible' fraction as found in Tessier's scheme was dropped – presumably because no iron and manganese oxides would be expected to be present - and replaced with a long extraction using 0.1M $Na_4P_2O_7$. This was deemed to yield organically complexed metals, which are considered, by Hsu and Lo (1999), to be relatively labile and potentially bioavailable. Subsequent extraction of the residue with 0.1M NaOH yielded metals deemed by Hsu and Lo to be organically bound. The researchers considered these metals to be relatively immobile and might not be readily bioavailable. Around 70%, of the copper present in the systems was to be found in these organic fractions. Over time organically complexed copper decreased slightly and the organically bound increased slightly. Fulvic acids (FA) were also removed from these extracts and their copper contents measured. FA-bound copper was seen to decrease from 99-100% of the extract at the start of composting to 72-77% in the mature product (presumably converting to humic acid bound copper) suggesting again that composting causes copper to stabilize. However, it can be said that with the exception of the sample taken at day 18 (end of the thermophilic stage) the sum of the organically bound and organically complexed copper changed very little over the course of the experiment. This is consistent with work outlined in their earlier paper (Hsu and Lo, 1999) in

which copper extracted from fraction four of the Tessier scheme, the 'organically bound' fraction – remained between 53 and 67%.

Increases in 'readily available' copper during the thermophilic phase of the composting of sewage sludge, with and without amendments of bauxite have also been observed (Qiao and Ho, 1997). Here copper was also seen to exist as predominantly organic forms a small proportion of which, it was concluded, was converted to carbonate bound and oxide bound forms. Stable complexes of fulvic acids were also found. These changes were a result of small increases in pH of the systems due to composting processes. Soluble carbon was also seen to increase and this was felt to confirm their findings. The addition of red mud (bauxite) substantially increased the number of inorganic oxide surfaces to which copper could bind and further increased pH values leading to the its precipitation. Red mud additions therefore inhibited the release of exchangeable forms of copper thus mitigating the effects of the thermophilic phase of the composting process. However, as the amounts involved were already small the addition of red mud barely had any effect on the overall speciation of the copper.

This pattern has not been seen by other workers. A dramatic decrease in dissolved organic carbon has been seen during the composting of the sludge produced at a Chinese waste water treatment plant (Zheng *et. al.,* 2004) attributable to microbial oxidation of the most labile forms and then subsequent volatilization.

During sequential extraction analyses changes in all fractions are usually observed, the most common one being a considerable increase in Tessier's 'residual' fraction (Zorpas *et. al.,* 2000, composting sewage sludge), most likely via the 'organically bound' fraction (Greenway and Song, 2002b, composting various green wastes) with corresponding decreases in this fraction or that described as 'HCl-extractable' (Pare *et. al.*, 1999, co-composting biosolids, woodchips and MSW). Petruzzelli's 1985 scheme, as used by Canarutto *et. al.* (1991) does not yield a 'residual' fraction. This scheme has only three stages, namely a water-soluble, a 1M KNO_3 extraction and finally a 1% EDTA extraction. Canarutto notes that there were decreases in copper extracted by the first two stages but an increase in copper removed by 1% EDTA from compost produced by the DANO process. There was though an overall decrease in concentration of all metals at the end of composting. This was attributed to the development of less extractable forms of metal such as those bound to humified organic matter. These presumably would have been detected in a 'residual fraction' if one had been produced and while the authors state that total metal content was measured, they do not report these results or comment on them.

These results are consistent with those of others. Gao *et. al.* (2005) found that most of the copper present in composting sewage sludge was in the 'organic' fraction but that over time it was, along with copper found in

earlier fractions, gradually converted into ‘residual’ forms. Szymanski *et. al.* (2005) also found that copper was concentrated in the less easily available fractions and became more so as composting age increased; Slightly different though were those results of Amir *et. al.* (2005) who found with Sposito’s extraction scheme that copper associated with the ‘carbonate bound’ as well as the ‘organically bound’ fraction decreased.

Decreases in copper in the earlier fractions extracted, often termed ‘easily exchangeable’ or ‘potentially exchangeable’ or ‘water soluble’, are usual (Greenway and Song, 2002b; Pare *et. al.*, 1999; Canarutto *et. al.,* 1991). Zorpas *et. al.* (2000), however, saw an increase in these forms of copper but attributed it to the breakdown of organic forms which then became extractable. A corresponding decrease in total copper present in the system as determined by a digestion procedure was also seen. This was found to be due to the uptake of copper by zeolites which had been added to the system at the start of composting and then removed before the start of the analysis.

Other workers (Zheng *et. al.,* 2004; Ciba *et. al.,*1997) when studying changing operational speciation of other metals have also subjected samples taken at various points in the composting process to SE schemes.

It is fair to say that, in the main, SE schemes have been applied to the mature product in order to fractionate the different chemical forms or

associations of the different metals present. Copper is consistently found to be predominantly extracted by those reagents believed to extract organically associated forms (Gao *et. al.,* 2005; Chwastowska and Sterlinska, 2000; Ciba *et. al.;* 1999; Chwastowska and Skalmowski, 1997; Tisdell and Breslin, 1995; Bourque *et. al.,* 1994;), often accounting for up to 90% or the copper present in the material (Chwastowska and Skalmowski, 1997). He *et. al.* (1995) applied their scheme to a range of composts obtained from various facilities across the US and claimed to separate the organically extractable from the organically bound. The sum of these two fractions also revealed that for most (though not all) of their samples, most of the copper is associated with organic forms.

Occasionally, the residual fraction is the dominant form in which copper is discovered. This may well be due to the presence of scrap metal, despite initial removal of large contaminants from the municipal waste feedstock (Prudent *et. al.,* 1993). Bourque *et. al.* (1994) also reported 'flyers' in their results – replicates much higher or lower than the others - as a result of the inclusion or exclusion of metallic fines. This may well account for those composts analysed by He *et. al.* (1995) which also demonstrated a predominance of copper in the residual fractions. Szymanski *et. al.* (2005) noted the existence of 'stray results' also caused by the occurrence of pieces of metallic copper not removed during the segregation process. It was not absolutely clear from the text whether or not these 'stray results' were connected with the residual or some other fraction though.

Planquart *et. al.* (1999) in France, having co-composted anaerobically digested sewage sludge with bark or green waste for either one or six months also found that most of the copper existed in residual forms. No explanation was proffered but as the total amounts present in the composts were much lower than France's permissible levels it could be hypothesized that it would therefore not take very long for organically bound forms of copper to be converted to residual ones. These workers also reported that reducible forms of copper exceeded those that were organically bound or 'oxidisable'. However, they used a modified version of the Tessier scheme in which they attempted to extract the humic fraction *before* the reducible one. This would, perhaps explain the apparent comparative lack of 'organic' forms.

In conclusion then, SE schemes have yielded a good deal of very interesting data though little of it has been used to actually measure bioavailability. So far only Planquart *et. al.* (1999) have attempted this and they found that uptake of copper by colza increased when the compost was added to acid soils. Much therefore remains to be done and it is likely that correlation with other speciation approaches, particularly non-destructive ones, coupled with bioavailability assessments may be a more productive and informative way forward.

1.10 Use of Cation and Anion Exchange Resins in Environmental Research

Ion exchange is the chemical reaction which takes place when an ion in solution exchanges with a similarly charged ion attached to a solid phase. In industrial situations these solid phases are particles of either inorganic zeolites or synthetic organic resins (Remco Engineering, date unknown). Such resins are used extensively in water softening and purification systems. These synthetic resins were first developed in the 1930's and their adsorptive properties investigated (Adams and Holmes, 1935). They are now used in preference to zeolites because they can be tailor-made to suit specific requirements and most of them are based on styrene, polymerized to produce long chains and then reacted with divinyl benzene to produce cross-linkages (Skogley and Dobermann, 1996). A schematic diagram of their formation is given in Fig. 1.5

In soil studies macroporous ion-exchange resins in the form of beads are those most commonly used. Choice of resin depends on the information required. Ion-exchange resins fall broadly into 2 classes – cation exchangers and anion exchangers (Table 1.3) although there is one other – that of the chelating resin, useful for the study of transition metals.

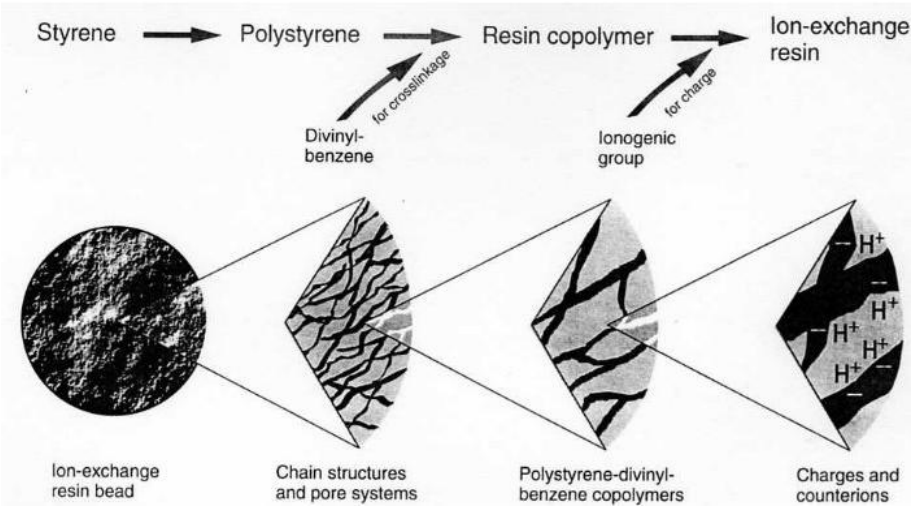


Fig. 1.5 Formation of ion-exchange resins and properties of a macroporous resin bead. (Source: Skogley and Dobermann, 1996).

Table 1.3 Resin Types Commonly Used in Agricultural and Environmental Research

(Source: Skogely and Dobermann, 1996; Dorfner, 1991)

Exchange Type	Commonly Used Active Groups	Sub-class	Nature	pH Range	Additional Comments
Cation	Sulfonic acid; carboxylic acid; hydroxide	Weakly acidic	Strong affinity for H^+	5-14	Appropriate for studies on plant nutrient uptake.
		Strongly acidic	Weak affinity for H^+	0-14	Appropriate for studies on release of cations from soils, cation mobility or bioavailability
Anion	Primary, secondary or tertiary amines most commonly functional groups	Weakly basic	Strong affinity for OH^-	0-9	
	Type I resins – trimethylamine groups Type II resins – dimethyl-β-hydroxyethylamine groups	Strongly basic	Weak affinity for OH^-	0-14	

These metals should, in theory, be able to be removed by cation exchangers. However, as their concentrations are often much lower than those of macronutrients like calcium and magnesium they are often unable to compete effectively for resin exchange sites. Chelating resins therefore overcome this problem by selectively extracting these metals by chelation (details of the nature of chelation are given in section 1.2).

Resin use in agriculture lies mainly within the area of research although this has now extended to routine soil testing of phosphorus (Hislop and Cooke, 1968). The range and extent to which these materials have been used in environmental and agricultural research has been thoroughly reviewed (Qian and Schoenau, 1997 and 2002; Skogley and Dobermann, 1996) and has been found to date back as far as 1942 (Schlenker, 1942) where they were employed as a source of nutrient ions in a sand culture system.

Early researches concentrated on the use of resins as sources of nutrient ions for plant growth, but in the main studies have concentrated on using them as ion sinks to assess macro nutrient bioavailability or their rates of release, the first paper in this vein being published in 1951 (Pratt, 1951).

Subsequent work has included amongst other things the determination of soil potassium (Salomon and Smith 1957) by adsorption onto Amberlite IR-120 resin (resin uptake of K correlated significantly with non-exchangeable K, total K and K uptake by hay); biologically available soil

phosphorus (Hedley *et. al.,* 1982) by batch extraction of soil with water plus Dowex-1 resin in a nylon bag; release characteristics of soil phosphorus (Curtin *et. al.,* 1987) and its relationship to other cations present in the system – increases in exchangeable calcium as a result of liming seemed to promote phosphorus release in a way not previously acknowledged; metal contaminants and their speciation in soils (Liang and Schoenau, 1996) by equilibrating soil suspensions with ion exchange resins of differing pH values; metal speciation in water (Cox *et. al.,* 1984) by Donnan dialysis across cation exchange membranes; labile metals in sediments (Beveridge *et. al.,* 1989) and solid waste leachates (Christensen and Lun, 1989); herbicide determination (Szmigielska *et. al.,* 1998 and Szmigielska and Schoenau, 1999) though with minimal success and even soil microorganisms (Hopkins *et. al.,* 1991a, b) where a chelating ion-exchange resin was used as an aid to the dispersal of soil in saline solution. A considerable amount of work has also taken place covering soil nitrogen mineralization and immobilisation (e.g. Bhogal *et. al.* ,1999; Zou *et. al.,* 1992; Johnson *et. al.,* 2005).

Much of this work has involved the use of resin beads. Sometimes resin in membrane form is used instead, the first instance of its use in agriculture being in reported in 1964 when anion-exchange membrane was used to extract soil phosphate (Saunders, 1964). Resins used are similar to those used in the manufacture of beads. They are extruded into sheets and combined with reinforcing material to provide stability and

strength (Skogley and Dobermann, 1996) and they are either cation, anion or bipolar.

Most work carried out is within batch systems i.e. the soil/sediment sample is shaken with an extractant and the resin which acts as an ion sink. As the resin helps to maintain a low analyte concentration in the extractant, further release of ions is promoted until equilibrium is eventually established (Sparks, 1987). The batch technique is thus only an adaptation of the conventional single step soil extraction procedure and thus retains its chief disadvantage, namely that results are operationally defined and may only be an indication of bioavailability. Results are dependent on soil/solution ratios, speed and duration of shaking during extraction, method of separation of the resin from the solution (Yang and Skogley, 1992) and removal of the analyte from the resin. Trapping of soil particles within resin pellets is also a major drawback (Skogley and Dobermann, 1996).

Diffusion-sensitive systems employ a different approach and effectively measure both the rates of release from and diffusion through the soil system by placing the resin in direct contact with the soil. Some have already been mentioned (Pratt, 1951; Curtin *et. al.,* 1987; Bhogal *et. al.,* 1999; Johnson *et. al.,* 2005). Yang *et. al.* (1991a) established that movement of K, P and S from the solutions of saturated soil pastes to mixed bed cation/anion resin capsules depended on diffusion controlled phenomena and concluded that interfacial ion exchange (film diffusion)

between the soil solution and the resin capsule was likely to be the mechanism involved. Subsequent work demonstrated the high dependency on temperature of these processes and confirmed using existing kinetic models that the processes were diffusion controlled, selective for nutrients, soil dependent and varied with soil chemical and physical characteristics (Yang *et. al.,* 1991b; Yang and Skogley, 1992). Accumulation by the resin capsules of K, P, S and NH_4-N was also related to ion activities of the solution while Ca and Mg were not and were believed to be dependent on mass flow.

Use of resins in these 'diffusion sensitive systems' provides an attractive alternative to the batch system in that this approach seeks to mimic the interactions of plant roots with their surrounding soil environment by providing a measure of the contribution of ion diffusion through the material being tested (Skogley and Dobermann, 1996). Thus a more realistic and sensitive test of nutrient phytoavailability is determined (Yang *et. al.,* 1991a). Sometimes resins are used as beads contained within a bag, or in pellet or capsule form, or as membranes. Mixtures of both cation and anion exchange resins can be used when in the bead or pellet form. Both forms rely on nutrient ion diffusion along a diffusion gradient to the sink in question which is usually infinitely large compared with the ionic strength of the solution and, whilst roots may effect changes to the soil rhizosphere the resin method is believed to indicate both the quantities of nutrients available and their supply rate (Yang *et. al.,*

1991a). Pellets are available commercially within the US from Unibest Inc. (http://www.unibestinc.com).

Use of resin in membrane form is also popular for use in diffusion sensitive systems. They are also thought to behave in a similar way to plant roots i.e. 'see what the roots see' and therefore predict the ion content of the rhizosphere. Their added ease of use of handling as compared with resin in gel or pellet form has enabled them to be used *in situ* in a wide range of soil types and conditions ranging from the arctic (Dormann, 2001) to the Mojave desert (Drohan *et. al.,* 2005) and to bush soils of the Yucatan peninsula in Mexico (Weisbach *et. al.,* 2002). Membranes are buried, causing minimal disturbance to the soil and left for periods of time appropriate to the researcher's aims and objectives and as they act as sinks for the ions under investigation the need for soil sampling, drying, grinding and sieving is removed. All that needs to be done is for the membrane (both cation and anion membranes are used) to be removed and its analyte(s) stripped off and determined. Membranes contained within plastic holders for enhanced user-friendliness are available for leasing from Western Ag Innovations Inc. of Saskatchewan, Canada (http://www.westernag.ca) and are known as PRS^{TM} probes, PRS standing for Plant Root Simulator because of the way in which the probes are said to exhibit the surface characteristics and nutrient sorption phenomena displayed by plant roots (Greer *et. al.,* 2003). Examples can also be found where membranes are used in laboratory situations

(Conder and Lanno, 2000; Conder *et. al.,* 2001) or pot trials (Montgomery *et. al.* 2005) but burial in the field is by far the most common.

Adsorption capacity of resin membranes is no where near as large as those for resin beads and may actually be quite small compared with the nutrient supply in the soil (Skogley and Dobermann, 1996). They will, therefore, only act as sinks for a short time and once the counterions available for exchange have run out the proportions of ions present on the membrane are determined by the concentrations and differing affinities of the ions in the soil solution itself (Skogley and Dobermann, 1996). They are therefore classified as 'dynamic exchangers' (Cooperbrand and Logan, 1994) and their results are believed to represent, as closely as possible, the behaviour of plant roots.

This approach has its limitations. It is crucial to know whether the membrane is acting as a sink, a dynamic exchanger or a combination of the two (Skogley and Dobermann, 1996). Research on nutrient supply in paddy fields demonstrated that results were also strongly influenced by resin type, amount of resin exposed to a given area or volume of soil, length of time of this exposure, analyte under investigation and whether the resin was used *in situ* in the field or under laboratory conditions (Dobermann *et. al.,* 1994). Large spatial variability across the field was also seen. Other constraining factors include soil temperature, moisture and the presence or absence of competing sinks (Sulewski *et. al.,* 2002).

What must also be borne in mind is that results are not directly comparable with conventional soil extraction techniques as in diffusion sensitive systems results are usually expressed as µg analyte per cm^2 of resin membrane and often also as a function of time i.e. µg cm^2 s^{-1}. As dynamic exchangers they are therefore a measurement of nutrient supply rates or dynamic fluxes to the sink in question under those particular circumstances (Greer *et. al.,* 2003).

Assessment of soil nutrient availability, particularly of phosphorus, is where exchange resin membranes are most often used. However, assessments of metal speciation, especially with environmental concerns in mind are also evaluated using this approach.

1.11 Use of Ion Exchange Resins in Heavy Metal Speciation Research (Diffusion Sensitive Systems, Solid Matrices)

Ion exchange resins have been used to determine heavy metal speciation in solid matrices in a number of cases (Table 1.4) but their use in diffusion sensitive systems (DSS) as discrete entities is by no means extensive. Resin membranes have been used in soils and, occasionally sediments but no examples of their application in composts have been found. Because of the differing kinetics involved in DSS systems (see section 1.9), their greater applicability to and successes in the determining of nutrient phytoavailability and soil fertility it is surprising that

Table 1.4

Heavy Metal Analyses Using Ion-Exchange Resins

Author	Analytes of Interest	Substrate	Resin(s) Used*	System Used***	Notes
Drohan *et. al.* 2005	Fe, Mn, Cu, Zn + Al	Floodplain soils of the Mojave Desert, Nevada, USA.	Cation PRS™ probes	DSS	Field based
Lee *et. al.* 2005	Cr (VI) and Cr (III)	Flooded soils	DOWEX M4159, - Cu saturated form	DSS	Phytotoxicity measured (rice seedlings).
Yu *et. al.* 2004	Cr	3 soils	Dowex M4195, -Cu saturated form	Batch	Soils spiked with Cr (VI)
Vulkan *et. al.* 2002	Cu, Zn, Pb, Fe, Mn, Cd + Ni	Air-dried and partially composted sludge, sandy soil, mix of the sludge and sandy soil	Na-saturated Dowex-50W cationic exchange resin Cl-form of Dowex macroporous resin-basic anionic exchanger.	Batch	Lab based
Conder *et. al.* 2001	Cd, Pb + Zn	Soil near Zn and Pb smelter	Anion PRS™ – DTPA saturated, - Na form	DSS	Soils amended or not with 1 of 2 biosolids or rock phosphate. Toxicity to earthworms measured. Lab based.
Lin *et. al.* 2001	Cd, Cu, Zn + Pb	Contaminated soil	Chelating resin membrane	DSS	Diffusion model produced
Conder and Lanno 2000	Cd, Pb + Zn	Artificial soil	Anion PRS™ – DTPA saturated, - Na form	DSS	Soils spiked with various heavy metal salts. Toxicity to earthworms measured. Lab based.
Tambasco *et. al.* 2000	Cu + Zn	Contaminated urban soils from Montreal + 1 forest soil	AEM – DTPA saturated, di-sodium form AEM – EDTA saturated, di-sodium form	Batch	Lab based. Phytoavailability assessed.
Agbenin *et. al.* 1999	Fe, Mn, Cu + Zn	Tropical soils	Mixed ion exchangers: Amberlite IRA-120 and Amberlite IRA-400 modified with Na forms of: Chloride; bicarbonate; fluoride; acetate; citrate or tartrate.	Batch	
Lombi and Gerzabek 1998	Cd, Cu + Zn	Soil amended and unamended with sewage sludge	Chelex-100, -Ca form	Batch	

Author	Analytes of Interest	Substrate	Resin(s) Used*	System Used***	Notes
Esnaola and Milan 1998	Cd, Ni, Pb, Mn, Zn + Cu	Contaminated urban Spanish soils.	All in –Na form: Chelating resins: Metalfix-Chelosolve (active groups tetraethylenpentaamine-pentaacetic acid) and Amberlite IRC-718 (active groups iminediacetate). Strongly acidic resin: Amberlite IRC-120 (active groups sulphonic acid). Weakly acidic resin: Amberlite DP-1 (active groups carboxylic acid).	Batch	Resin in bags.
Fotovat and Naidu 1997	Zn + Cu	11 soils	Cation exchange resin	Batch	Resin method compared favourably with the geochemical model MINTEQA2.
Lee *et. al.* 1996	Cd	9 Taiwanese agricultural soils	Chelex resin membrane – Ca saturated	DSS	4 environmentally contaminated soils, 5 artificially contaminated agricultural soils .
Liang and Schoenau 1996	Cd, Cr, Ni + Pb	Soil set 1: Cultivated soils: 1 loamy sand and 1 clay loam. Soil set 2: Contaminated urban soils	CEM – H^+ form CEM – Na form AEM – DTPA saturated, - H^+ form AEM – DTPA saturated, - Na form.	Batch	Cultivated soils artificially contaminated with metals of interest.
Liang and Schoenau 1995	Cd, Cr, Ni + Pb	Light soil and a heavy soil	AEM – DTPA saturated, - Na form.	DSS	Soils spiked with metals of interest. Results from membrane burial compared with conventional DTPA extraction. Phytotoxicity also assessed.
Lee and Zheng 1994	Cd, Cu + Pb	9 Taiwanese agricultural soils	Chelex resin membrane – Ca saturated	Batch	4 contaminated soils, 5 uncontaminated soils.

Author	Analytes of Interest	Substrate	Resin(s) Used*, **	System Used ***	Notes
Liang and Schoenau 1994	Cd, Cr, Ni + Pb	Light soil and a heavy soil	AEM – DTPA saturated, - Na form.	DSS	Soils spiked with metals of interest. Results from membrane burial compared with plant uptake and phytotoxicity.
Tejowulan *et. al.* 1994	Cu, Zn, Mn + Fe	32 Saskatchewan soils and 14 Indonesian soils	AEM – DTPA saturated, - Na form. AEM – EDTA saturated, - Na form	Batch and DSS	Conventional DTPA and EDTA extractions compared with AEM batch and burial procedures. Uptake by Canola assessed.
Lee and Zheng 1993	Cd	8 Taiwananese agricultural soils	Chelex resin membrane – Ca saturated	Batch	4 contaminated soils 4 spiked soils Uptake by wheat seedlings measured
McLaughlin *et. al.* 1993	Al	Soil	Cation exchange resin membranes – NH_4^+ form Anion exchange resin membranes – Cl^- form	Batch	Plant nutrients also analysed and correlated with results from conventional extractions. Al did not correlate well though.
Jing and Logan 1991	Cd	17 anaerobically digested sludge samples	Chelex 100 – Na saturated	Batch	Resin Cd correlated with uptake by Sudax
Beveridge *et. al.* 1989	Cu, Pb, Zn + Cd	1 Tidal creek sediment 1 estuarine sediment	10 different cation exchange resins	Batch	Both sediments contaminated by local pollution.
Camerlynck and Kiekens 1982	Fe, Mn, Zn, Cu, Pb, Cd + Ni	Air dried soil	Cation exchanger: CM-Sephadex-C25, - Na form Anion exchanger: DEAE-Sephadex A-25, - acetate form	Column	Speciation of metals according to size separation.

* Where AEM = Anion exchange membranes, CEM = Cation exchange membranes
** Unless stated as being in membrane form, resins are used either as pellets or as a gel.
*** DSS = Diffusion Sensitive System

there are so few examples reported. It is therefore difficult to make comparisons with data from more conventional techniques.

Diffusion sensitive systems provide information regarding sorption/desorption processes in a way that batch systems (both with and without resins) never can. Conventional extractions often remove far larger amounts of the available pool than the plants do, thus leading to doubts over the suitability of their use (Tejowulan *et. al.,* 1994). Use of exchange resins, particularly chelating ones serve to overcome this problem where heavy metals at trace element or micronutrient levels are involved. Tejowulan *et. al.* (1994) in an investigation of a range of soils with varying levels of micronutrients is the earliest example. A diffusion sensitive approach was compared with both a resin batch extraction as well as conventional extractions and compared with uptake by canola (*Brassica napus* L. cv. Westar). Strips of anion exchange membranes were converted to di-sodium forms of EDTA or DTPA (subsequently termed AEM-EDTA and AEM-DTPA) thus transforming them into chelating resin membranes and then used in both the DSS approach and the resin batch extraction procedures. All methods were significantly related to plant uptake for Cu, Zn, Mn and Fe and all generated precise results. Average values for copper extraction from the diffusion sensitive system were 3.2% and 3.7% for the resin batch extraction procedure. It was unfortunate, however that no information was provided on the relative performances of the two differently treated resin membranes in these two situations in the DSS (burial) procedure. The researchers concluded that resins pre-treated with chelating agents gave a positive approach to assessing micronutrient availability and

using resin membranes in a burial method was a particularly simple, rapid and convenient approach.

Liang and Schoenau (1994 and 1995) in their work on 2 artificially contaminated Saskatchewan soils also used chelating resin membranes to assess micronutrient availability. Membrane extracted cadmium, chromium and nickel significantly correlated for all three plants and measurement of nickel was an excellent predictor of bioavailability with uptake by oats, radish and lettuce. Lead however did not correlate at all and reflected its extremely low uptake from the soils. Membrane extracted metals also correlated well with spike rate and conventional DTPA extractions (Liang and Schoenau, 1995). Again, membrane burial (a DSS approach) was lauded as being a simple and convenient method that also has the advantage of including the diffusion component present in the soil. Tejowulan *et. al.* (1994) and Liang and Schoenau (1995) have therefore successfully related resin membrane uptake of metals from Saskatchewan and Indonesian soils whose metal contents ranged from the deficient to the phytotoxic, to metal uptake by a number of different plants under laboratory conditions. Similarly, others (Lee *et. al.,* 1996 and Lee *et. al.,* 2005) have also established the viability of using chelating resin membrane as an indicator of phytoavailability. Heavy metal determination of soils by chelating resin membranes was used in an attempt to predict earthworm mortality rates (Conder and Lanno, 2000; Conder *et. al.,* 2001) but with no success, the authors concluding that this was owing to differing mechanisms of uptake between earthworms and plant roots. However, PRS^{TM} uptake of cadmium and zinc in their single metal test

appeared to increase with increasing metal content. Precision of the PRS^{TM} probe method should therefore have been assessed. Further tests with increased replication (more probes per soil sample and more soil replicates) and a range of burial times would achieve this and establish whether the probes were acting as sinks or dynamic exchangers.

All these examples are of chelating resin membranes used within the laboratory. Only one reported instance of use for heavy metal determination in the field has been found. Western Ag Innovations' PRS^{TM} probes (anion and cation) were used *in situ* in Nevada in the Mojave Desert (Drohan *et. al.* 2005) over 2 seasons (wet and dry). Over time copper removal was seen to plateau whilst zinc was seen to decrease. However, as chelating PRS^{TM} probes were not used nor conventional extractions on soil samples carried out by way of a comparison, these results must stand alone and cannot be properly interpreted. Other reports of results for trace metals removal from other DSS by cation exchange have not been found elsewhere in the literature.

Thus, very few occurrences in the literature exist where chelating anion exchange resin membranes have been used to determine heavy metals and their likely phytoavailability. There is therefore considerable scope for their use in determining heavy metals in composts. No occurrences of their use in this manner within composting systems has been found and much more work needs to be done on all forms of solid environmental matrices before this approach would be regarded as a standard for measuring metals. As they

have not been put to use in metal speciation in either whole composts or composting systems there still remains a deficit of knowledge in this area.

1.12 Ion Exchange Resins and Sequential Extractions – Comparing the Two Techniques for Metals Analysis.

No paper exists giving an account of metal speciation in any kind of solid matrix as determined by both of these two methods. Nevertheless, the smelter contaminated soil at Blackwell, Oklahoma as used by Conder *et. al.* (2001) in their bioavailability experiment was used earlier in an investigation of the effects of the same amendments on metal uptake by and phytotoxicity in lettuce and effects of lowering pH. Soil metal levels were measured using a potentially bioavailable assessment sequential extraction scheme or PBASE method (Basta *et. al.,* 2001).

Conder *et. al.* (2001) had found that pH appeared to be more important than organic matter in immobilising the metals studied since lime-stabilised biosolids (LSB) which had a low organic matter content and high pH were more successful at reducing earthworm toxicity than anaerobically digested municipal biosolids (SS). This helped to support the earlier findings of Basta *et. al.* (2001). Reductions in cadmium phytoavailability were achieved by the additions of LSB (possibly by being adsorbed to or precipitated as carbonates) and were consistent with cadmium extracted by the first reagent in their extraction scheme [20ml of 0.5M $Ca(NO_3)_2$] which had earlier been found to be highly correlated with phytoavailability in lettuce (Basta and Gradwohl,

2000). Phytotoxic levels of zinc as measured by the $Ca(NO_3)_2$ fraction were also reduced by the addition of these amendments and phytotoxic levels steadily increased when pH levels were reduced.

PRS^{TM} probes were able to demonstrate that LSB significantly lowered the amount of bioavailable Zn as compared with both the control and rock phosphate (RP) treated samples but not the SS samples (Conder *et. al.,* 2001). The control and RP samples were also not found to be significantly different from each other. Stage 1 in the PBASE method also extracted significantly less Zn from the LSB treated Blackwell soil compared with the control and RP treatments but in this case the SS treatment was also significantly different from the LSB treatment. Under this sequential extraction scheme the SS was also not found to have significantly different extractable Zn levels as compared with the control. Only on these two points do results from the PRS^{TM} probes differ from those of the sequential extraction scheme. However, if measures were taken to ensure adequate precision was obtained in the use of these probes then these differences might not have arisen.

Overall, information on the comparative performances of these two approaches is lacking.

Chapter Two

Compost Trial 1.

Results from the work described in this chapter were presented at the Rural Renewal 2000 Conference 1998, Harper Adams University College, 15 - 21 July 1998. A copy of the paper from the conference proceedings (Cruxton and Keeling, 1998) can be found in Appendix I. A.A. Keeling wrote the summary and introduction, whilst this author (then under her maiden name of Cruxton) wrote the methods, results and discussion.

Introduction

Copper is a common contaminant in various waste streams, arising from activities such as metal-working and tannery processes, and whilst a valuable micronutrient when present at low levels, it is toxic to plants and microorganisms at higher ones (Cruxton and Keeling, 1998). In particular, copper has been shown to have a toxic effect on nitrogen-fixing bacteria at low concentrations (Keeling and Cater, 1998; Cruxton and Keeling, 1998). Green waste composting by local authorities has increased in recent years and even more so in response to the UK Government's 'Waste Strategy 2000' directive (DEFRA, 2000a and b). It therefore seemed appropriate to determine the effect of composting processes on the chemical forms of copper, as

determined using a sequential extraction procedure, in composted green waste originating from grass clippings and pine sawdust.

2.1 Materials and Methods

2.1.1 Compost Production

Two waste materials were used for composting. These were locally obtained grass clippings and pine sawdust, a by-product of the construction industry. The grass clippings were immediately refrigerated until required (approximately 3 ½ hours) and then sorted to remove any dead leaves, twigs and other contaminants. The sawdust was passed through a 5.6mm test sieve as manufactured by Endecotts, UK to remove large pieces of woody material and also checked for the presence of contaminants.

Prepared materials were combined at a grass to pine sawdust ratio of 1:1.5 (on a wet weight basis) to give an estimated C:N ratio of 41:1 and a dry matter content of 61.5% (± 1 s.d. of 0.97%). Into each of 20 wide-necked screw capped 500ml capacity HDPE bottles were weighed 100g of this fresh, bulk material and these were then supplemented, on a wet weight basis, with a copper (II) acetate (BDH, Analar grade) solution at rates of 0 mg kg^{-1} Cu ; 500 mg kg^{-1} Cu; 1000 mg kg^{-1} Cu and 1500 mg kg^{-1} Cu. These rates were equivalent to 0, 813, 1626 and 2439 mg kg^{-1} Cu on a dry matter basis. Copper in the di-acetate form was chosen because of its high solubility. When in solution, copper acetate complexes would be expected to be able to cross

bacterial cell membranes but then owing to their low stability, dissociate when encountering chelating groups within the cell (Keeling and Cater, 1998), thus potentially inducing toxic effects.

Five replicate bottles were prepared for each treatment level and, with the additional copper solutions having been added, all the materials for composting had a mean resultant moisture content of 47.5% (± 1 s.d. of 0.91%) and a mean dry matter content of 53.5% (±0.92%). The lids were replaced and the contents thoroughly mixed and 1 bottle for each treatment level was stored at -20^{o}C until required for analysis (t=0 days).

Under normal composting conditions, temperatures at the centre of a pile or windrow can exceed 70^{o}C and be maintained for significant periods of time due to the insulating properties of the surrounding material. Weekly turning ensures that all the material attains this temperature at some point over the composting period and is therefore sanitised. Due to the small scale nature of the experiment the volume of materials involved was insufficient to generate the heat that would otherwise have naturally occurred due to microbial action. To this end the bottles were placed in random positions in an incubator and the temperature of the incubator was adjusted at different times to mimic the changes normally seen in the centre of a large windrow system.

The bottles were shaken and aerated every 2-5 days to keep the systems aerobic and were removed (one replicate bottle per treatment level per sampling day) on days 7, 21, 179 and 238, weighed and then stored at

-20^{o}C until required. At the start of the experiment the incubator was set at a temperature of 35^{o}C and then was adjusted to 50^{o}C on day 7, 65^{o}C on day 21 and reduced to 55^{o}C on day 37 which was then maintained until day 238 at which point it was felt that the materials had undergone a sufficient period of maturation. On day 31 it was noted that 5 of the bottles (one at each level of copper) were not degrading as well as the others. Humification appeared to have stopped and there was a distinct alcoholic smell. This was probably due to a lack of headspace in the vessel making aeration difficult (despite the fact that the headspace in all the bottles was the same) and leading to anaerobic conditions. To remedy the situation the contents of these bottles were transferred to larger, 1000ml HDPE screw-capped bottles and not sampled until day 238.

2.1.2 Analyses

Bottles were defrosted, their lids were removed and the bottles then placed in an oven at 70^{o}C to dry their contents to a constant mass. The contents were then removed and ground to pass a 1mm sieve (Cyclotec mill, Tecator, Sweden). These samples were then analysed in triplicate for ash and volatile solids by loss on ignition at 500°C and also for copper using a seven step sequential extraction scheme (Table 2.1) the stages of which were based on the work of a number of authors. Briefly, the ground samples (2g ± 0.001g) were weighed out into 60ml Nalgene^{TM}, oak ridge polysulphone tubes which had with sealing caps (Fraser and Lum, 1983) and subjected to the sequential analysis scheme using 30ml extractant at each stage and shaken

Table 2.1

Seven Step Sequential Extraction Scheme

Fraction	Reagent + Conditions	Author	Nominal Phase Extracted
1	Deionised water, 16 hours @ 23^{o}C		Water soluble
2	0.5M $Ca(NO_3)_2$, 16 hours @ 23^{o}C	Miller *et. al.* (1986)	Neutral salt extractable/exchangeable
3	0.1M Sodium acetate @ pH 5.0, 5 hours @ 23^{o}C	Fraser and Lum, (1983)	Surface oxide and carbonate bound
4	0.04M Hydroxylamine-hydrochloride in 25% acetic acid, 6 hours @ 90^{o}C with occasional agitation	Fraser and Lum, (1983)	Reducible
5	0.1M $K_4P_2O_7$, pH 10, 16 hours @ 23^{o}C	Bascomb (1968)	Organically bound
6	0.1M EDTA, 16 hours @ 23^{o}C	Miller *et. al.* (1986)	Chelate extractable
7	Ashing at 500^{o}C and digestion in 20ml 69% HNO_3	Dry Ashing method (AOAC, 1990)	Residual/non-extractable

on a Gallenkamp horizontal shaker for the required period of time. The hydroxylamine hydrochloride extract was the exception. At this stage the tubes were placed in a water bath to maintain the necessary 90˚C and occasionally agitated by hand. After each extraction the tubes were centrifuged at 15,000 rpm in a Beckman Avanti 30 centrifuge at 4^{o}C for 30 minutes and the supernatants decanted. The decanted supernatants were filtered via a Whatman 541 filter paper and then stored in 50ml HDPE containers with screw caps. The pellets of material left after each extraction were subjected to a water wash to remove any residual extractant (Miller *et. al.* 1986). This was achieved by the addition of 20ml deionised water, shaking for 15 minutes, centrifuging at 15,000 rpm and then removal and discarding of the water wash by decanting. After the removal of every extractant or water wash the weights of the tubes were recorded in order to calculate the volume of reagent or water wash left behind.

Water and calcium nitrate extracts were acidified to pH<2.0 with concentrated HNO_3. Copper in the extracts was determined by flame-Atomic Absorption Spectrophotometry (AAS) at 324.7nm, using matrix matched standards. Details for converting raw AAS data to levels of extracted copper are given in Appendix II.

Separate whole samples were analysed for total copper by subjecting them to a standard digestion in concentrated nitric acid (AOAC, 1990). Briefly, the sample remaining in the extraction tube was quantitatively

transferred using deionised water from a wash bottle to an evaporating basin. The contents were dried at 100°C to remove the water then ashed at 500°C overnight in a muffle furnace. The resulting ash was then digested in 20ml of 69% nitric acid and then diluted to 50ml with deionised water. This solution was then filtered via a Whatman 541 filter paper and stored in 50ml HDPE containers with screw caps until they could be analysed for copper by AAS as described.

All solutions were prepared using deionised water; all plastic containers were soaked overnight in 69% nitric acid to remove any contamination that may have existed as a result of previous work and all chemicals used were Analar grade or better.

2.2 Results

Moisture Content, Dry Matter, Volatile Solids and Ash Contents

Results for moisture content, dry matters, volatile solids, and ash are given in Table 2.2. Moisture contents (and hence, dry matter contents) at t = 0 days were, as expected, very similar for all treatment levels, showing that all materials and copper solutions had been mixed in a very consistent manner. Few changes in moisture levels were seen after 7 days of composting. By t = 179 and 238 days the situation was very different and substantial losses in moisture were seen. The 0 copper treatment, for example, had decreased from 46.6% moisture to just

Table 2.2 Moisture, Dry Matter, Volatile Solids and Ash Content of Materials at Sampling Times

Sampling Time (Days)	Sample Treatment (Wet Weight Basis mg/kg)	Sample Treatment (Dry Weight Basis mg/kg)	Original Wet weight materials g	Mass Wet Materials at Sampling g	% Mass Loss	% Moisture of Materials at Sampling	% Dry Matter of Materials at Sampling	Dry Matter Content of Bottles as % of Dry Matter Content at t = 0 Days***	Loss On Ignition			Total Cu Extracted		Total Cu by Digestion	
									% Volatile Solids	% Ash	95% CI	mg/kg (dry basis)	95% CI	mg/kg (dry basis)	95% CI
0	0Cu	0	130.1	130.1	0.0	**46.6	** 53.4	98.5	90.4	9.6	1.29	491	165	450	28
	500 Cu	813	129.5	129.5	0.0	46.8	53.2	98.9	90.0	10.0	0.50	1346	209	1090	295
	1000 Cu	1626	130.0	130.0	0.0	48.0	52.0	101.0	90.5	9.5	0.29	2659	453	1944	495
	1500 Cu	2439	130.1	130.1	0.0	48.5	51.5	101.7	89.9	10.1	1.01	3339	659	2776	287
7	0Cu	0	130.5	129.9	0.5	45.5	54.5	95.3	90.8	9.2	1.42	424	353	429	85
	500 Cu	813	130.2	129.7	0.4	47.5	52.5	99.5	90.2	9.8	0.98	1480	402	1094	493
	1000 Cu	1626	131.0	130.5	0.4	48.3	51.7	100.9	90.1	9.9	1.34	2378	296	1922	160
	1500 Cu	2439	132.6	132.1	0.4	48.0	52.0	99.6	90.1	9.9	0.18	3357	165	2793	191
21	0Cu	0	130.4	*	*	*	*	96.9	89.4	10.6	1.43	503	141	586	80
	500 Cu	813	130.1	*	*	*	*	98.3	90.5	9.5	0.51	1594	427	1169	76
	1000 Cu	1626	131.0	*	*	*	*	100.9	89.9	10.1	0.66	2453	86	1860	175
	1500 Cu	2439	133.3	*	*	*	*	104.0	89.2	10.8	1.84	3281	196	2681	531
179	0Cu	0	131.3	68.1	48.1	11.0	89.0	87.0	89.0	11.0	1.38	586	101	438	171
	500 Cu	813	131.7	70.7	46.3	12.3	87.7	92.7	88.9	11.1	0.52	1449	603	1079	96
	1000 Cu	1626	132.3	106.1	19.8	47.2	52.8	88.3	88.4	11.6	0.58	2514	252	2097	75
	1500 Cu	2439	133.3	80.8	39.3	31.5	68.5	94.5	88.6	11.4	1.06	3473	42	2590	122
238	0Cu	0	131.5	76.4	41.9	28.5	71.5	97.1	88.9	11.1	0.87	541	72	418	58
	500 Cu	813	114.7	84.0	26.8	28.6	71.4	98.8	90.6	9.4	0.55	1449	115	1088	40
	1000 Cu	1626	130.8	86.4	34.0	36.1	63.9	88.3	87.0	13.0	6.81	2331	28	1799	268
	1500 Cu	2439	132.1	80.6	39.0	25.8	74.2	86.4	88.8	11.2	0.39	2946	165	2375	151

* Data not available

** Mean % moisture content for 0 Days all treatments = 47.5% (± 1 s.d. of 0.91%); mean dry matter content for 0 Days = 51.5% (± 1 s.d. of 0.92%).

*** Calculated dry matter content for all treatments at t = 0 days was 61.5g ± 2.41g (i.e. ± 3.92%) at $p<0.05$.

11.0%. Solids were therefore in greater proportion (89%). Only the 1000 mg kg^{-1} treatment had managed to retain most of its moisture after 179 days (47.2%). The 0 and 500 mg kg^{-1} bottles sampled at 238 days contained proportionally less dry matter (and proportionally more moisture) than bottles sampled at 179 days for the same treatments, whilst the reverse was true for the 1000 and 1500 mg kg^{-1} treatment bottles.

The dry matter content of the bottles changed over time and appeared to decrease by as much as 15.5% for the 1500 mg kg^{-1} treatment (significantly different at $p<0.05$). However, most of the changes seen were not significant. Only between t = 0 days and 179 for the 0 copper treatment; 0, 7 and 21 between 179 and 238 days for the 1000 mg kg^{-1} treatment and between 0 and 238 days for the 1500 treatment were there significant differences. Very small changes were seen in the proportions of volatile solids and ash in the samples. An apparent decrease was seen in the ash content of the 500 mg kg^{-1} treatment between 179 and 238 days was seen. This most likely reflects incomplete degradation of the material. The overall trend though was of a very small increase in the ash content of the samples and a very small decrease in volatile solids. Over the 238 days the 0 mg kg^{-1} increased its ash content from 9.58% to 11.1%; the 1000 mg kg^{-1} from 9.54% to 13.0% and the 1500 mg kg^{-1} treatment, from 10.1 to 11.2%. However, no significant differences were seen between any of the treatments at the different sampling times.

Overall then, the vast majority of changes in mass that occurred were due to a loss of moisture rather than actual decomposition of materials.

Sequential Extractions

Data for each extraction are presented in both mg kg^{-1} and as a percentage of the total amount of copper extracted from each sample (the percentage share) in Tables 2.3 and 2.4. Figures 2.1 – 2.4 show the changes in copper partitioning over the composting period for all treatments and individual extractions both in terms of the amounts extracted and the percentage share. The data was not corrected for loss of mass of materials.

Water Extracts

The 0 mg kg^{-1} Cu treatment showed a slight increase in extractable amount extracted to 6.80%) during the first 7 days of the experiment (Tables 2.3 and 2.4). During the next 14 days this decreased to 14.58 mg kg^{-1} (2.92% of the total amount extracted) and then remained steady until day 179. However, a marked increase in the levels of water extractable copper between 179 and 238 days was then seen, with the amount of copper extracted increasing from 15.48 mg kg^{-1} to 80.38 mg kg^{-1}, with the initial percentage share of 2.65% increasing to 14.88%. The 500 mg kg^{-1} Cu treatment followed a very similar trend, though with larger amounts of copper being extracted and with water extractable copper making up a greater proportion of the total amount of copper extracted by the

Table 2.3 Copper Extracted in Each Fraction (mg/kg) According to Sampling Time and Overall Recovery

Time Days	Treatment (mg kg^{-1} on wet weight basis)	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5		Fraction 6		Fraction 7		Total Extracted		Total by Digestion		%Cu Recovered by Extraction
		Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	Cu mg/kg	SE	
0	0Cu	6.59	0.07	-0.44	0.11	3.97	0.16	1.14	0.03	0.74	0.21	45.11	2.00	434.19	36.43	491.31	38.30	450.19	6.51	108.95
	500 Cu	169.00	6.20	14.19	0.37	345.75	13.24	40.16	1.51	93.68	7.64	297.89	10.35	379.65	23.87	1346.01	48.53	1089.80	68.53	113.47
	1000 Cu	509.30	22.48	75.42	10.37	787.20	13.45	243.45	17.42	279.30	23.27	276.82	28.75	487.49	112.04	2658.97	105.36	1944.16	115.14	137.29
	1500 Cu	655.90	41.38	119.66	15.43	1127.54	12.36	258.16	18.66	434.89	14.10	242.52	7.87	500.42	95.09	3339.10	153.21	2776.28	66.86	120.68
7	0Cu	26.99	0.21	2.18	0.50	12.08	1.38	3.80	0.09	8.63	1.15	60.26	7.45	310.45	71.68	424.39	82.20	428.76	19.74	101.17
	500 Cu	270.15	7.68	23.43	1.05	241.25	22.97	29.86	3.11	138.46	14.96	410.83	44.45	366.21	21.74	1480.19	93.48	1093.67	114.64	139.86
	1000 Cu	438.65	6.93	54.24	7.46	579.12	9.79	238.70	14.57	336.40	23.03	339.25	22.32	391.52	43.53	2377.88	68.79	1922.28	37.23	123.70
	1500 Cu	799.90	5.32	111.70	6.03	991.38	11.39	276.28	25.56	531.05	21.20	320.26	5.12	326.67	23.30	3357.24	38.43	2792.56	44.45	120.26
21	0Cu	14.58	0.02	0.79	0.70	7.94	0.60	2.98	0.38	11.04	0.43	86.91	9.18	379.00	28.09	503.24	32.79	585.80	18.60	86.41
	500 Cu	245.00	3.18	22.51	0.44	281.78	21.78	96.83	43.35	131.07	8.05	478.13	37.95	339.14	57.87	1594.47	99.40	1168.62	17.68	136.75
	1000 Cu	530.25	20.92	58.41	3.12	510.66	6.83	240.26	11.23	324.95	7.15	408.44	30.65	380.40	44.49	2453.37	20.04	1860.41	40.63	132.03
	1500 Cu	642.60	37.11	87.56	4.62	917.33	11.14	343.19	16.93	530.94	16.54	370.52	5.68	388.68	14.15	3280.82	45.70	2681.48	123.50	122.98
179	0Cu	15.48	0.08	-1.17	0.03	13.11	0.76	21.10	0.52	27.79	1.60	191.07	10.66	318.37	26.42	585.77	23.54	437.91	39.67	136.91
	500 Cu	225.15	1.04	38.71	1.47	109.77	12.34	326.75	14.84	141.95	16.54	370.25	13.93	236.85	124.97	1449.42	140.19	1079.17	22.43	134.49
	1000 Cu	168.80	2.26	7.10	0.44	278.94	6.77	516.92	20.75	423.29	30.34	712.25	4.57	407.10	19.69	2514.40	58.65	2096.93	17.36	119.88
	1500 Cu	375.10	8.86	156.67	13.05	590.15	39.37	888.82	104.76	480.03	15.90	595.56	12.25	386.29	45.29	3472.62	9.71	2589.52	28.49	134.13
238	0Cu	80.38	0.88	22.21	2.19	34.55	0.79	111.80	9.71	38.71	1.46	139.16	3.56	114.68	7.56	541.49	16.74	418.26	13.58	129.61
	500 Cu	316.80	1.05	96.47	2.92	99.56	8.04	363.74	5.89	89.35	4.48	289.30	4.46	193.99	33.40	1449.20	26.75	1087.77	9.40	133.27
	1000 Cu	555.55	6.77	335.86	26.29	79.72	3.63	428.35	15.79	91.27	0.66	573.51	6.73	266.70	15.59	2330.96	6.60	1798.68	62.22	129.92
	1500 Cu	456.55	23.11	753.35	25.36	153.44	10.48	504.15	20.20	167.88	15.79	606.96	8.92	303.21	23.65	2945.55	38.47	2375.32	35.07	124.05

*Key to Fractions: 1 = Water Extract; 2 = Calcium Nitrate Extract; 3 = Sodium Acetate Extract; 4 = Hydroxylamine Hydrochloride; 5 = Potassium Di-Phosphate Extract; 6 = EDTA Extract; 7 = Residual Fraction.

Table 2.4 Copper Extracted in Each Fraction As Percentage of Total Amount Extracted During the Sequential Extraction Scheme. According to Sampling Time.

Time Days	Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5		Fraction 6		Fraction 7		Total Extracted mg/kg
		average %	SE	average %	SE	average %	SE	average %	SE	average %	SE	average %	SE	average %	SE	
0	0Cu	1.36	0.09	-0.09	0.02	0.82	0.09	0.23	0.01	0.16	0.05	9.23	0.31	88.29	0.52	482.46
	500 Cu	14.39	1.91	1.20	0.12	29.17	2.74	3.45	0.56	8.11	1.68	25.24	2.90	28.18	0.76	1394.54
	1000 Cu	19.17	0.65	2.88	0.51	29.66	0.77	9.16	0.57	10.52	0.86	10.52	1.51	18.09	3.51	2461.42
	1500 Cu	19.63	0.73	3.62	0.58	33.89	1.41	7.72	0.23	13.04	0.18	7.31	0.56	14.79	2.16	3096.03
7	0Cu	6.80	1.13	0.51	0.03	2.93	0.25	0.95	0.14	2.08	0.15	14.58	1.08	72.15	2.64	377.08
	500 Cu	18.37	1.07	1.60	0.13	16.27	0.89	2.06	0.32	9.30	0.50	27.59	1.30	24.81	1.17	1625.28
	1000 Cu	18.49	0.80	2.28	0.30	24.37	0.29	10.05	0.63	14.12	0.65	14.29	1.05	16.40	1.35	2349.95
	1500 Cu	23.83	0.27	3.32	0.14	29.53	0.02	8.22	0.69	15.84	0.81	9.54	0.11	9.72	0.62	3356.94
21	0Cu	2.92	0.19	0.14	0.13	1.58	0.11	0.60	0.08	2.20	0.08	17.28	1.60	75.27	1.74	496.57
	500 Cu	15.48	0.96	1.42	0.10	17.89	2.15	5.96	2.49	8.25	0.46	29.96	1.15	21.03	2.55	1396.35
	1000 Cu	21.61	0.83	2.38	0.14	20.82	0.43	9.79	0.47	13.24	0.22	16.66	1.34	15.48	1.73	2446.85
	1500 Cu	19.58	1.03	2.67	0.11	27.98	0.69	10.46	0.50	16.18	0.38	11.29	0.06	11.84	0.27	3350.41
179	0Cu	2.65	0.10	-0.20	0.01	2.24	0.14	3.61	0.11	4.74	0.14	32.74	2.44	54.21	2.82	563.59
	500 Cu	15.84	1.59	2.73	0.33	7.65	0.82	22.84	1.64	10.02	1.63	26.05	2.79	14.87	8.02	1177.17
	1000 Cu	6.72	0.21	0.28	0.02	11.10	0.28	20.56	0.75	16.80	0.80	28.35	0.49	16.19	0.63	2631.02
	1500 Cu	10.80	0.28	4.51	0.36	16.99	1.09	25.61	3.06	13.82	0.43	17.15	0.32	11.12	1.29	3483.41
238	0Cu	14.88	0.61	4.10	0.37	6.39	0.19	20.58	1.14	7.18	0.45	25.75	1.04	21.13	0.74	573.90
	500 Cu	21.87	0.36	6.67	0.32	6.89	0.67	25.11	0.33	6.18	0.43	19.97	0.33	13.31	2.09	1454.84
	1000 Cu	23.83	0.32	14.40	1.09	3.42	0.16	18.38	0.67	3.92	0.02	24.61	0.34	11.45	0.70	2330.79
	1500 Cu	15.52	0.99	25.58	0.82	5.20	0.29	17.10	0.48	5.70	0.51	20.61	0.05	10.29	0.76	2978.51

*Key to Fractions: 1 = Water Extract; 2 = Calcium Nitrate Extract; 3 = Sodium Acetate Extract; 4 = Hydroxylamine Chloride; 5 = Potassium Di-Phosphate Extract; 6 = EDTA Extract; 7 = Residual Fraction.

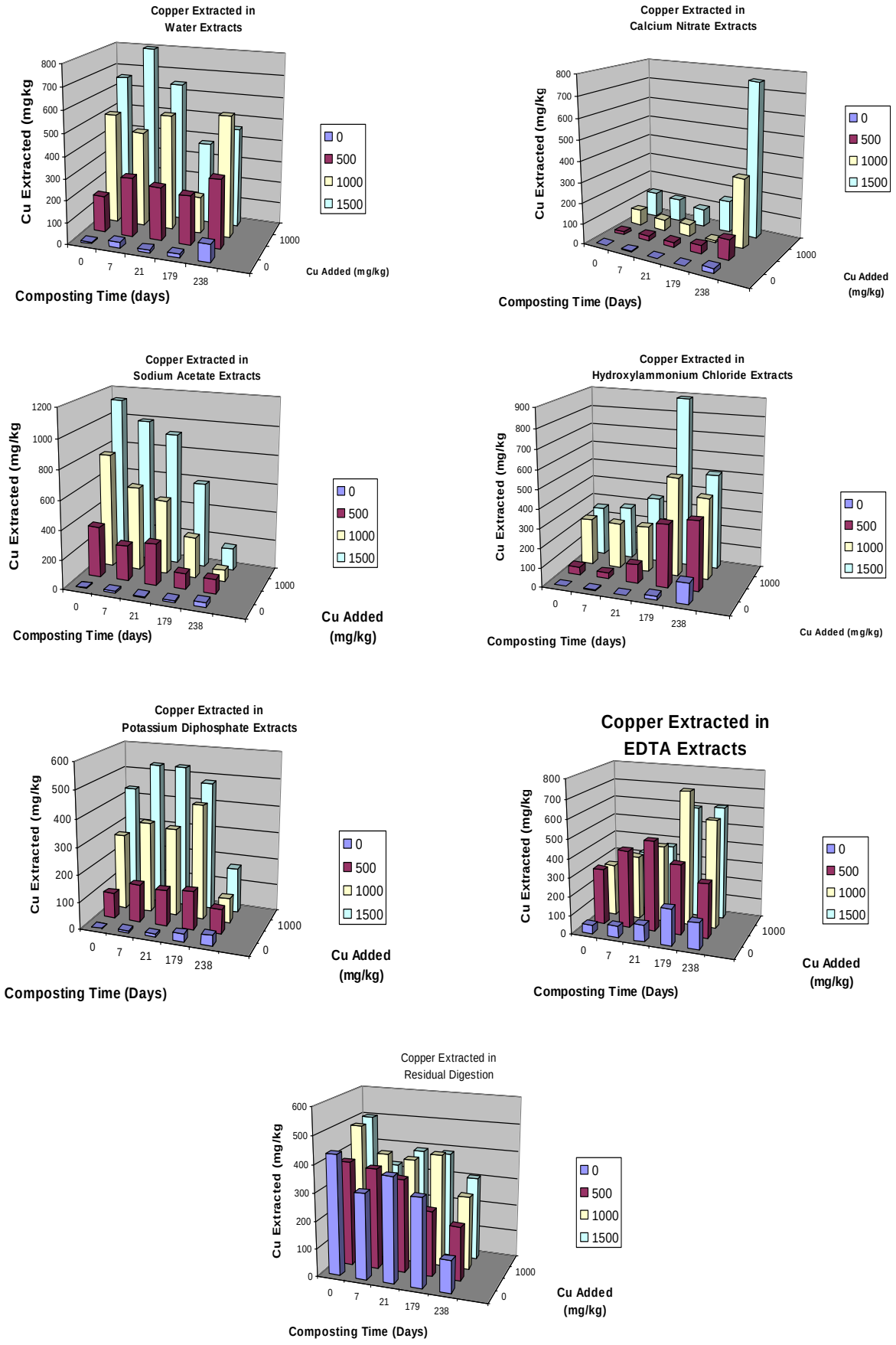


Fig. 2.1 Copper Extracted ($mgkg^{-1}$) from Composting Materials in the Individual Fractions of a Seven-Step Sequential Extraction Scheme.

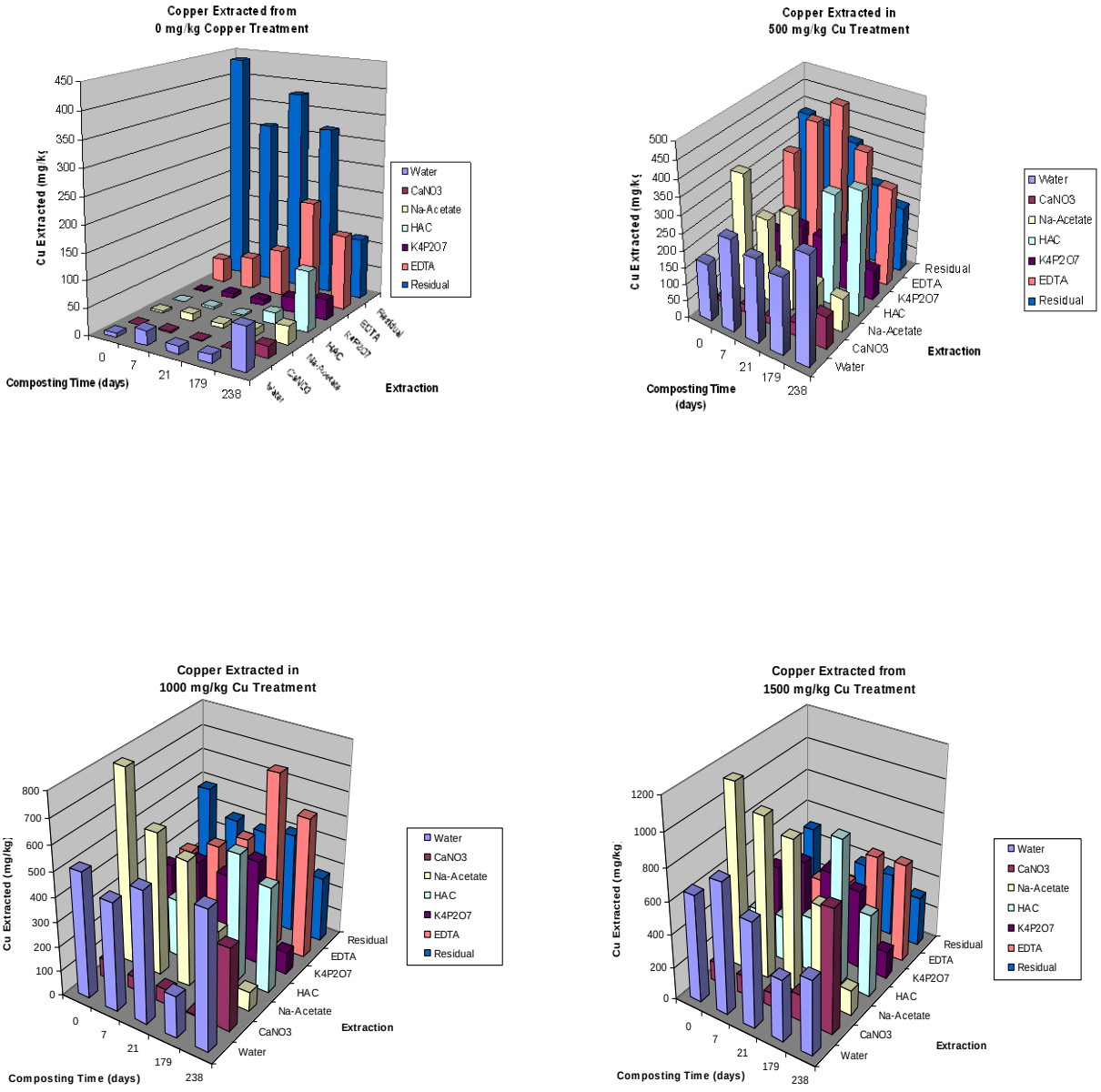


Fig 2.2 Copper Extracted ($mgkg^{-1}$) from Copper Acetate Treated Composting Materials

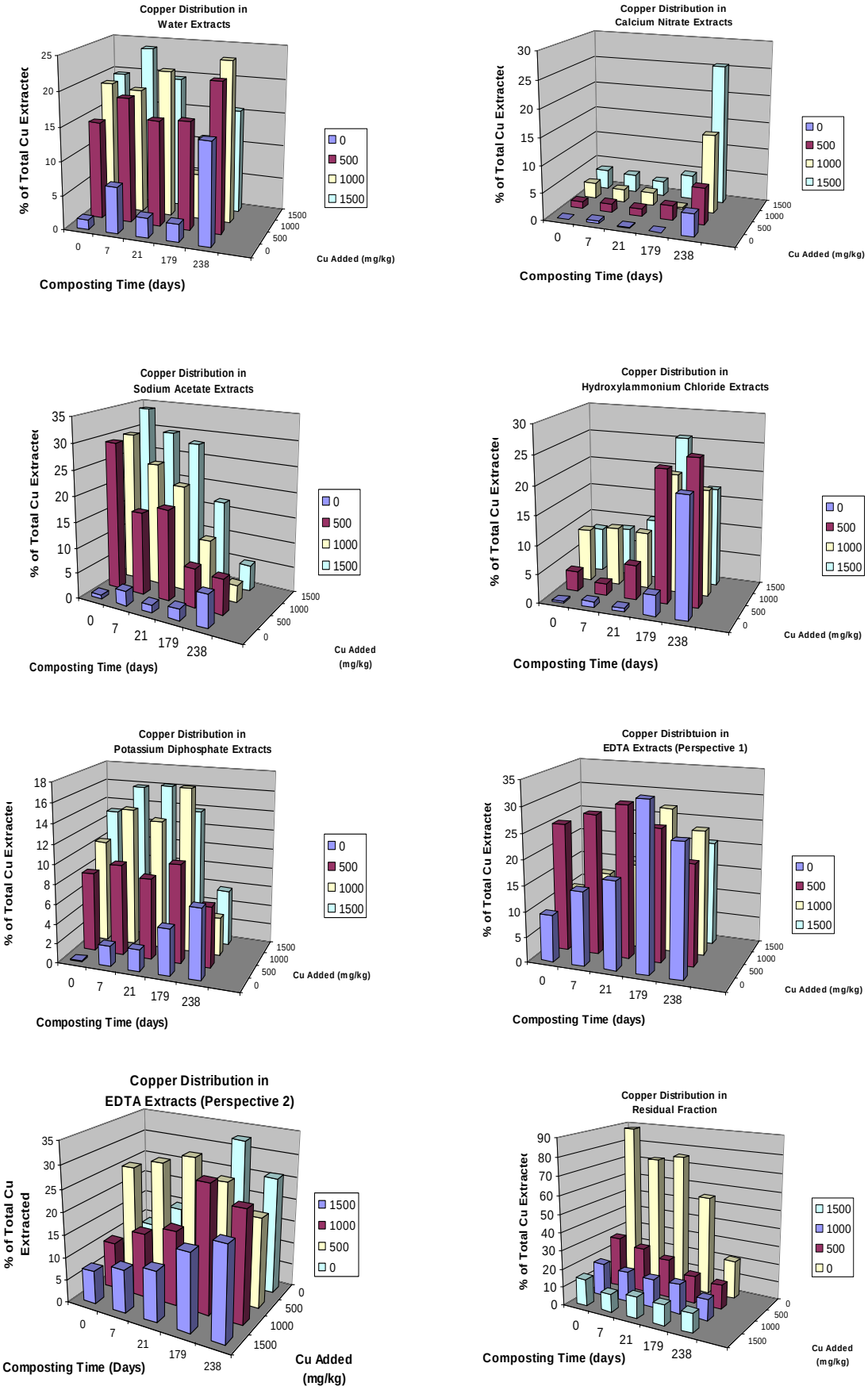


Fig. 2.3 Copper Extracted as a Percentage Share from Composting Materials in the Individual Fractions of a Seven-Step Sequential Extraction Scheme.

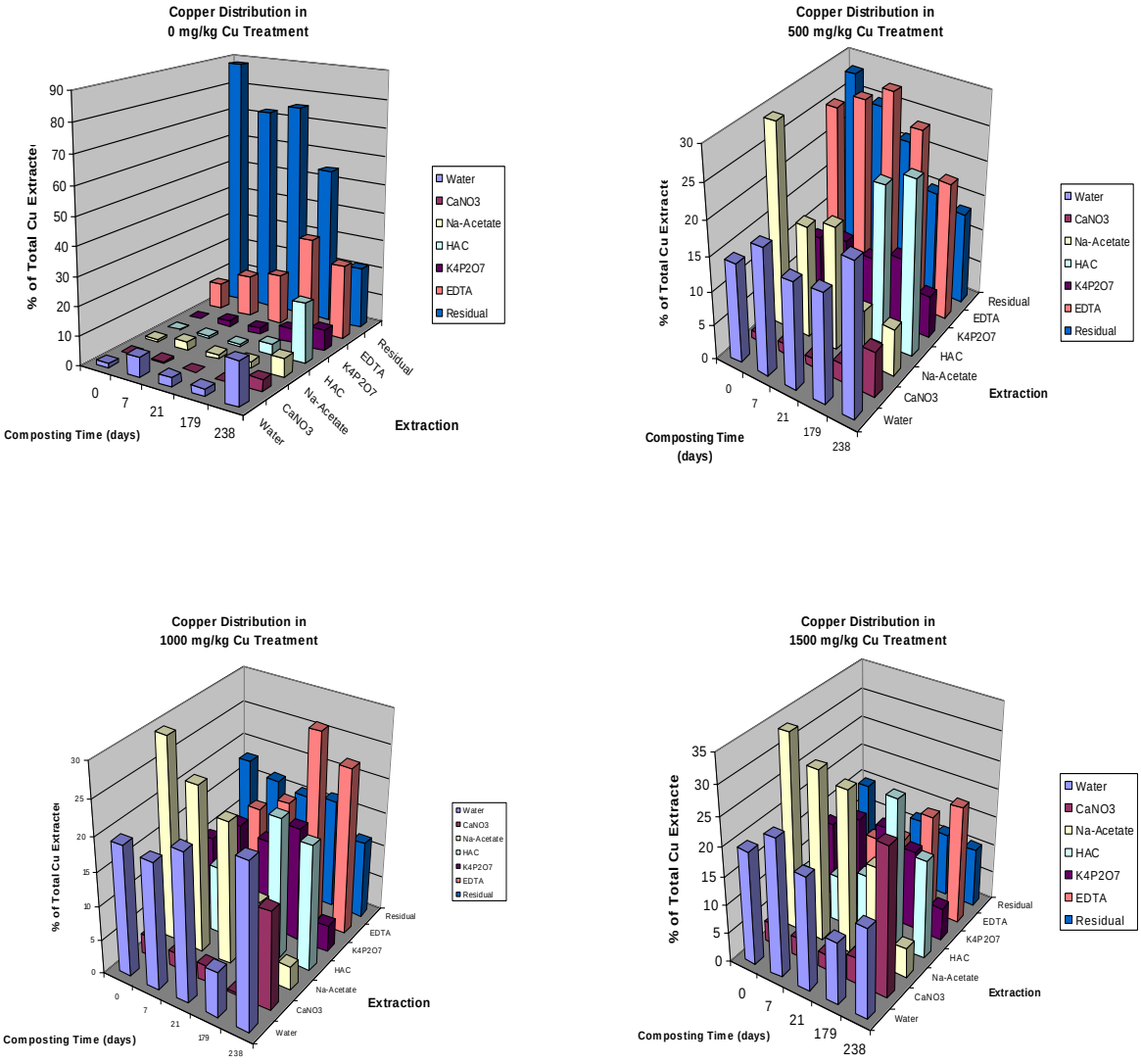


Fig 2.4 Copper Extracted as a Percentage Share from Copper Acetate Treated Composting Materials

sequential extraction scheme. However, fluctuating levels of copper meant that no clear trend could be established in the next highest copper treatment (1000 mg kg^{-1} treatment). The 1500 mg kg^{-1} treatment, like the 0 and 500 mg kg^{-1}, also saw an increase in water extractable copper during the first 7 days. However, between days 7 and 179, water extractable copper decreased from 799.9 mg kg^{-1} to 375 mg kg^{-1}. Extractable copper then increased slightly to 456.55 mg kg^{-1}.

In general, it was found that for almost all points in time, the amount of water extractable copper increased with increases in the level of copper treatment. Within-sample variation for all these extractions was low. Relative standard deviation values for extractable copper expressed in mg kg^{-1} were all below 11%.

0.5M Calcium Nitrate Extracts

For all treatment levels there appeared to be little change in the amounts of copper extracted for the first 179 days and generally, the greater the copper treatment, the more copper was extracted in this fraction, although sometimes there was no discernable difference between the 1000 and 1500 mg kg^{-1} treatments. This was also true for the percentage share in this fraction. After day 179, calcium nitrate extractable copper for the 0, 500, 1000 and 1500 mg kg^{-1} treatments respectively was 22.21, 96.47, 335.9 and 753.4 mg kg^{-1} which corresponded to 4.1, 6.67, 14.40 and 25.58% of the total amounts of copper extracted from each of these samples. Within-sample variation was not as low

as for the water extracts with relative standard deviations for extractable copper often exceeding 20%. For the 0 mg kg^{-1} treatment, very small amounts of extractable copper (less than 1 mg kg^{-1}) meant that variation was much higher and at day 179 this resulted in a relative standard deviation of 153%.

1.0M Sodium Acetate Extracts

Copper extracted from the 0 mg kg^{-1} treatment follows a pattern very similar to that seen in the water extracts. Extractable copper increased during the first 7 days of the experiment from 3.97 mg kg^{-1} to 12.08 mg kg^{-1} (0.82 to 2.93% of the total extractable amount). This decreased during the following 14 days but increased again over the rest of the composting period to 34.55 mg kg^{-1} (6.39% of the total copper extracted).

In contrast, the other treatments displayed strong, steady decreases throughout the course of the whole experiment in both the amounts and the percentage share of the copper extracted.

By day 238 of the experiment the amounts of copper extracted by the 1.0M sodium acetate were very different in each treatment and ranged from 34.55 mg kg^{-1} for the 0 mg kg^{-1} to 153.440 mg kg^{-1} for the 1500 mg kg^{-1}. However, when expressed as a proportion of the total extractable amount, the treatments were all very similar with values ranging from only 3.42% to 6.89%.

0.04M Hydroxylamine Hydrochloride in 25% Acetic Acid

Very few changes were seen in any of the treatments during the first 179 days of the experiment. Copper extracted from the 0 mg kg^{-1} treatment during the first 21 days was very low – less than 4 mg kg^{-1} (or less than 1% of the total amount of copper extracted by the sequential extraction scheme). By day 179 this had increased to 21.10 mg kg^{-1} (or 3.61% of the total extractable copper) and by the end of the experiment this had increased much further to 111.80 mg kg^{-1} (20.58% of the total extractable amount).

For the 500, 1000 and 1500 mg kg^{-1} treatments though, the most significant changes occurred between day 21 and day 179. Overall increases in hydroxylamine hydrochloride extractable copper were seen in all three cases. Between day 179 and day 238 fewer dramatic changes were observed. However, it must be emphasised that it is not possible to say what is happening to the rates of change in copper extractability between sampling times, only what the overall changes are. The 500 mg kg^{-1} treatment continued to increase but only by 37 mg kg^{-1} (from 326.75 mg kg^{-1} to 363.74 mg kg^{-1}). The two highest treatment levels though decreased in extractable copper. The 1000 mg kg^{-1} treatment decreased from 516.92 mg kg^{-1} to 428.35 mg kg^{-1}, whilst the 1500 mg kg^{-1} treatment saw a greater decrease in extractable copper - from 888.82 mg kg^{-1} to 504.15 mg kg^{-1}. This may be due to large within-sample variability (relative standard deviation was 20.7%) or, possibly, conversion of the copper to other forms.

The amounts extracted from each treatment from the bottles sampled at day 179 were all significantly different from each other at the 95% confidence interval (i.e. differences were greater than 1 standard deviation) and were in the order of 0 mg kg^{-1} < 500 mg kg^{-1} < 1000 mg kg^{-1} < 1500 mg kg^{-1}. However, as a proportion of the total amount extracted by the sequential extraction scheme, the trend is somewhat different and instead follows the order of 0 mg kg^{-1} < 500 mg kg^{-1} = 1000 mg kg^{-1} = 1500 mg kg^{-1}. By day 238 the trend in the proportion of copper extracted by the hydroxylamine hydrochloride had shifted to 0 mg kg^{-1} < 500 mg kg^{-1} > 1000 mg kg^{-1} = 1500 mg kg^{-1} with the 500 mg kg^{-1} treatment containing a greater proportion of extractable copper than all the others, the reasons for which are unclear. It should also be noted that the 0 mg kg^{-1} treatment was also not significantly different from the 1000 and the 1500 mg kg^{-1} treatments. It appears that the overall trend was for hydroxylamine hydrochloride extractable copper to reach equilibrium at around 17-20% of the total extractable copper.

0.1M Potassium di-Phosphate Extracts

This reagent is said to extract metals which are bound by specific absorption to organics (McLaren and Crawford, 1973). The trends for changes in copper extracted in this fraction are not as clear as those observed in some of the other extracts. For the 0 mg kg^{-1} copper treatment there was a steady increase in the amount of copper extracted, rising from 0.74 mg kg^{-1} at the start of the experiment to 38.71 mg kg^{-1} at the end. In terms of copper

extracted as a proportion of the total amount removed by the scheme, this too increased, rising from 0.16% to 7.18%.

For the other treatments, increases in the amounts of copper extracted also occurred. For the 500 and 1500 mg kg^{-1} treatments this was during the first 7 days of the experiment (though as a percentage share of the copper removed by the sequential extraction scheme this remained unchanged until day 179 for the 500 mg kg^{-1} treatment). Extractable copper in the 1000 mg kg^{-1} treatment did not increase until day 179; before this, copper levels remained steady. Pronounced decreases in extractable copper were then seen after day 179 in the 500 and 1000 $mgkg^{-1}$ treatments, whilst for the 1500 mg kg^{-1} treatment decreases took place earlier – between 21 and 179 days.

0.1M EDTA Extracts

All treatment levels demonstrated continuous increases over time in the amounts of EDTA extractable copper. For the 0, 1000 and 1500 mg kg^{-1} treatments these increases took place between 0 and 179 days whilst for the 500 mg kg^{-1} treatment they occurred only between 0 and 21 days. With the exception of the 1500 mg kg^{-1} treatment which appeared to plateau (the increase of 11.4 mg kg^{-1} as seen between day 179 and day 238 was not proven to be significant at the 95% confidence interval), EDTA extractable copper then decreased during the rest of the composting process. It is possible that had the experiment continued then a decrease in EDTA

extractable copper might also have been seen in the 1500 mg kg^{-1} treatment. These patterns of extraction were also mirrored by changes in the proportion of copper extracted by EDTA.

Residual Digestion

The general trend amongst all the treatment levels is one of a reduction in the amounts of residual copper. However, as a result of very high within-sample variability (e.g. the relative standard deviation for the 0 mg kg^{-1} treatment at 7 days was 40.0%) this can only be reliably established for decreases in the amounts of residual copper between day 179 and day 238 for the 0, 1000 and 1500 mg kg^{-1} treatments. For the 500 mg kg^{-1} treatment no significant differences could be established for any of the data at any of the sampling times. Within-sample variability for this treatment was, at times, much greater – the relative standard deviation for this treatment at day 179 was 91.4%. The data also indicates that the amounts of residual copper were the same for each treatment at the start of the experiment and thereafter for each sampling point for the first 179 days of the experiment clearly demonstrating that copper is not easily or quickly converted to these non-extractable forms. This is in marked contrast to all the other extracts, all of which had widely differing amounts of copper, depending on the treatment level. By 238 days amounts of residual copper now appeared to differ according to treatment level in the order: 0 mg kg^{-1} < 500 mg kg^{-1} < 1000 mg kg^{-1} < 1500 mg kg^{-1}. However, the large within-sample variability meant that this was not proven.

Despite this, data for residual copper expressed as a percentage of the total amount extracted by the sequential extraction procedure was less variable, enabling more conclusions to be drawn. At 0 days residual copper accounted for 88.3% of the copper within the 0 $mgkg^{-1}$ treatment. Still, within 179 days (approximately six months) this proportion had decreased to 54.2% of the total extractable amount and still further to only 21.1% by day 238, just 59 days (approximately 2 months) later. These decreases in residual copper over the first 179 days correspond with pronounced increases in EDTA extractable forms and much smaller increases in potassium di-phosphate forms over the same period. Over the final phase of the experiment (day 179 to 238), the residual fraction continued to decrease. At the same time the EDTA also decreased, exhibiting a reduction in copper of 51.9 $mgkg^{-1}$ (139 $mgkg^{-1}$ on day 238 down from 191 mg kg^{-1} on day 179). This corresponded to a decrease from 32.7% on day 179 to 25.8% of the total extractable amount on day 238, a difference of 7 percentage points. Importantly, these decreases corresponded with significant increases in copper extracted in the water, calcium nitrate, sodium acetate and hydroxylamine hydrochloride fractions, all believed to determine readily and potentially phytoavailable forms.

In the artificially contaminated treatments residual copper made up a much lower proportion of the overall copper present. Residual copper made up 28% of the total copper extracted from the 500 mg kg^{-1} treatment, 18.1% in the 1000 mg kg^{-1} treatment and 14.8% in the 1500 mg kg^{-1} treatment. Over the course of the experiment the trend was one of a reduction in residual copper and by 238 days residual copper expressed as a percentage share of the total

extractable amount, being p 21.1%, 13.3%, 11.5% and 10.3% of the total copper removed by the sequential extraction scheme from the 0 mg kg^{-1}, 500 mg kg^{-1}, 1000 mg kg^{-1} and 1500 mg kg^{-1} treatments respectively. The rate of change in residual copper is therefore very large for the 0 mg kg^{-1} treatment, whilst for the other treatments changes are far less marked. Nevertheless, had the experiment continued it is possible that all treatments might have contained the same proportion of residual copper.

Copper Recovery

The sum totals of copper recovered in all the extractions for each sample were compared with copper as determined by total acid digestion so that the efficiency of the extraction scheme could be calculated (Fig. 2.5). Owing to the lack of sample replication, average recoveries were calculated by randomly pairing the sum totals of copper extracted by the extraction scheme from each of the 3 within-sample replicates with the results of copper determined by total digestion of another 3 within-sample replicates. This approach, though not ideal, did at least ensure that an estimation of average variability could be calculated. In most instances the sum of all the fractions for each sample, differed greatly when compared with copper determined in the whole samples by acid digestion. Average recoveries for the 0 mg kg^{-1} treatment from the samples taken at 0, 7 and 21 days were 109; 101 and 86.4% respectively and samples from the 500 mg kg^{-1} treatment at 0 and 7 days were 114 and 140%

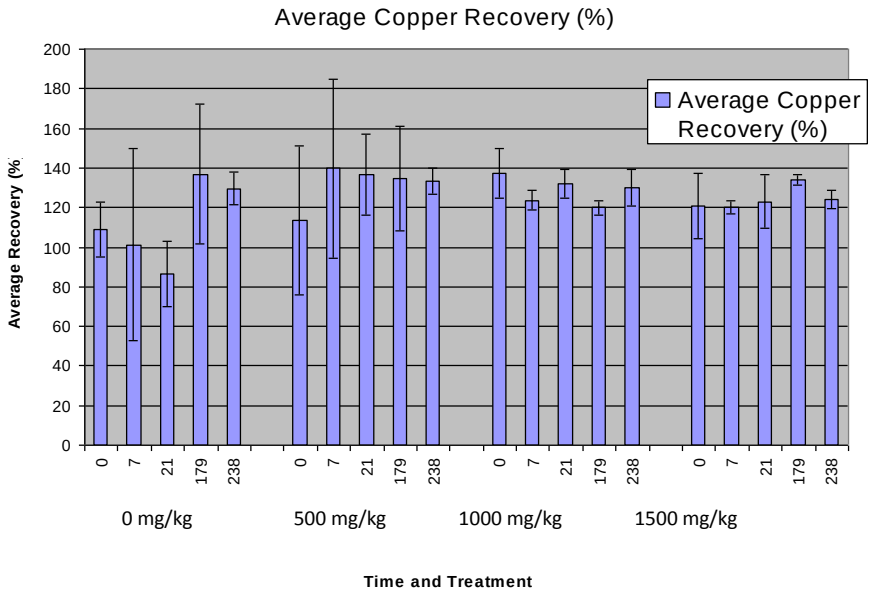


Fig. 2.5 Average Recovery of Copper at Each Sampling Time by the Seven-Step Sequential Extraction Scheme.

respectively and these did not differ significantly from 100% at the 95% confidence interval. For all other samples the average recoveries were significantly higher than 100%. Such high values illustrated the incomplete nature of the digestion of the whole samples by the acid, although they might also be due to the large number of extractions involved (Ho and Qiao, 1998).

Within-Sample Variability

Regression analysis of extracted copper with the percent relative standard deviation (%RSD) values for all extracts yielded an R^2 value of 0.021 (Fig. 2.6). This demonstrated that there was no relationship between the amount of copper extracted and variability within samples. Within-sample variability for amounts of copper extracted ranged from very low (%RSD for the 1500 $mgkg^-$

^{1} at 179 days for the total amount extracted was 0.48%) to extremely high (% RSD for the 0 $mgkg^{-1}$ at 21 days for the calcium nitrate extraction was 153%). The frequency of values for each 1% range of % RSD values is given in Appendix III. Values of less than 3% account for 20% of all values, those less than 11% account for 60% of all values, whilst all those less than18% account for 80% of all values. Values for %RSD between 18% and 24% account for a further 12.14% of all the values. The rest of the values, those above 24% RSD, account for no more than 8% of all values. Data appear to follow a normal distribution curve if these values which exceed 24% are discounted.

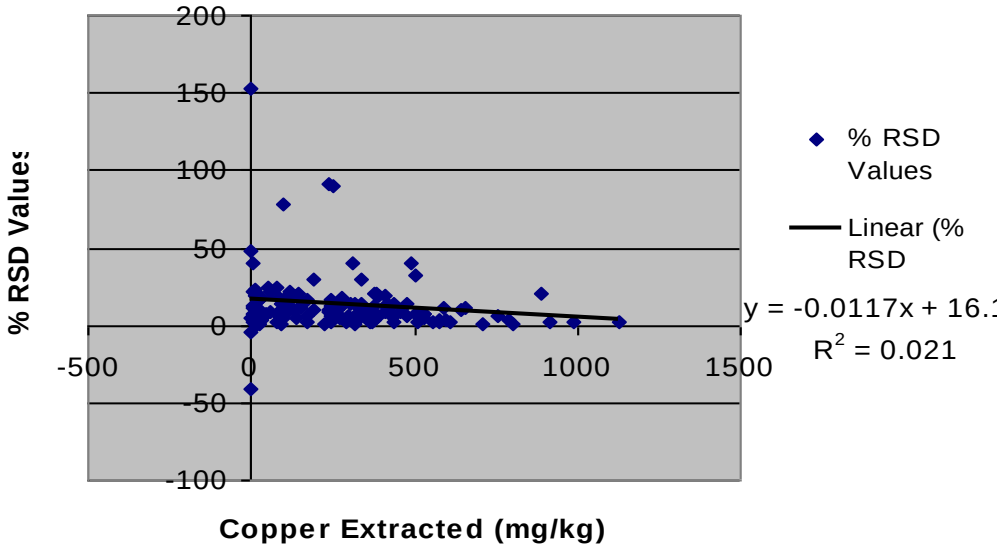


Fig. 2.6 Regression Analysis of Extracted Copper versus % Relative Standard Deviation Values.

Although there is a high degree of within-sample variability (40% of all %RSD values are greater than 11%) this is not inconsistent with the findings of other workers. Chwastowska and Sterlinska (2000) found that %RSD values for copper extracted from municipal solid waste compost by the BCR 3-step

sequential extraction scheme were very varied and ranged from 3.6-23.0% while mean and standard deviation figures reported by Tisdell and Breslin (1995) for copper in sequentially extracted fractions obtained from two municipal solid waste composts generated %RSD figures ranging from 0 to 79%. Such variability is attributed to problems with separating the solution from the material after extraction because of the tendency of the compost particles to float (Chwastowska and Sterlinska, 2000); the large numbers of manual manipulations inherent in the execution of sequential extraction schemes are also a major factor. With such high reported %RSD values it is therefore safe to conclude that the quality of the data collected in this trial matches those of other researchers and is likely to be as reliable as is possible to achieve.

2.3 Discussion

0 mg kg^{-1} Treatment

This material behaved quite differently to the 500, 1000 and 1500 mg kg^{-1} treatments and will therefore be considered separately. As most of the copper (88.29%) native to the starting materials at the beginning of the composting process was found in the residual fraction of the sequential extraction scheme, it should be assumed that this is unavailable for uptake by plants; the copper only being released into solution by the most aggressive acid digestion. However, it is clear from the results that the composting process began to alter these starting materials in terms of extractable copper. Residual

copper decreased from 88.29% to 54.2% of the total extractable amount during the first 179 days along with corresponding increases in EDTA extractable copper. EDTA extraction is believed to determine organically complexed metals (Amir *et. al.* 2005) and therefore can determine metals which are potentially available for plant uptake. After 179 days EDTA extractable copper decreased whilst extraction of copper in the earlier stages of the sequential scheme then started to dominate. In fact over the course of the experiment copper became increasingly extractable earlier on in the sequential extraction scheme. The experiment therefore clearly demonstrated that, with these materials, residual copper decreased and phytoavailable copper increased due to the composting processes.

Few workers, who have investigated changes over time in heavy metal operational speciation in composting materials, have studied the composting of green wastes. Those that have done so have reported somewhat different trends to those described here. Greenway and Song (2002b), using the Community Bureau of Reference (BCR) scheme, with an additional step included to determine residual forms, reported organically bound copper as being the dominant form throughout the composting process. Copper extracted in the earlier steps of the sequential extraction was seen to decrease over time and this corresponded with increases in residual forms of copper. Ho and Qiao (1998) in their investigations of the effects of bauxite on the distribution of heavy metals during the composting of municipal solid waste (MSW), produced an ‘artificial’ MSW to use as a control sample, namely a mixture of grass clippings and sawdust. They investigated changes

over 20 days, taking samples at the start and the end of the experiment but only studied changes in chromium speciation.

All other research has focussed on sewage sludge or MSW composting but with rarely any attempt to define the constituents of the municipal solid waste. Prudent *et. al.* (1993), described in detail the nature of the constituent parts of reconstituted municipal solid waste used at a composting plant in Cavaillon, France. Only on one occasion, was the makeup of the waste referred to and this was to state that the composition of the MSW was dependent on the source of the materials which is, in turn, a function of both season and location (He *et. al.* 1995).

Increases in EDTA extractable copper between day 0 and day 179 are attributable to the complexation of the metal with ligands formed as a result of the degradation (humification) of the materials. As compost matures and stabilizes humic substances are formed which become increasingly aromatic (and thus more stable), having a high molecular weight and many oxygen-containing functional groups, all having a high affinity for heavy metals (Petruzzelli 1996). Zorpas *et. al.* (2000) saw increases in copper in the earlier fractions of their scheme and attributed it to the breakdown of organic forms which then became extractable. This would account for the decrease seen in EDTA extractable copper after day 179 and increases in water, calcium nitrate, sodium acetate, hydroxylamine hydrochloride and also potassium di-phosphate forms of copper.

Residual forms of copper have been seen on occasion to be dominant. The presence of metallic fines explained why copper was found mainly in the residual fraction of MSW derived compost (Bourque *et. al.,* 1994) and research into the speciation of copper in 3 day-old and 6 month-old MSW compost (Prudent *et al.*, 1993) also showed that the majority of the native copper was bound within the residual fraction of both the compost and the constituent parts that made up the initial starting materials. Here, organics made up only 24% of the whole, while paper and cardboard, plastics, glasses, wood and metallics accounted for 56% of the materials, the copper in each being found predominantly in the residual fraction. Scrap metal was therefore attributed as the main source of the residual copper (Prudent *et. al.* 1993). Prudent *et. al.* also found that when these particles were dissolved they were then taken up by the residual fraction, thus immobilizing the metal and increasing the proportion of copper held there. However, as results for this grass and sawdust study demonstrated an overall release from this fraction this behaviour has not occurred in this study. These differences in behaviour are therefore accounted for by differences in starting materials. Levels of native copper were already high - in the order of 500 mg kg^{-1} which may be due to pre-treatment of the wood with copper-chromate-arsenate (CCA). This is applied under pressure to timber used in joists and floorboards as an insecticide to prevent infestation by pests such as the woodworm and also as a fungicide to prevent rotting. The use of CCA is common practise within the timber industry.

Availability of metals bound to humic substances are generally considered to be low and therefore pose no (or negligible) threat to the environment. However, this cannot be considered to be a foregone conclusion (Massiani and Domeizel, 1996). This is because the interactions between humic substances and metal ions are very complex and the bonding strengths between the two are so easily changed by pH, Eh (oxidising/reducing conditions) and the humification of the materials themselves. In this study, conversion from organic and residual forms to those extractable by both water and a neutral salt solution (in this case, 0.5M $CaNO_3$), identified as easily available to plants and micro-organisms (Petruzelli *et. al.* 1989; Miller *et. al.* 1986) has been demonstrated. The end product would have failed WRAP's PAS 100 Specification for Composted Materials (WRAP, 2005), which stipulates an upper limit of 200 mg copper per kg of composted material and a target of < 50 mg kg^{-1}. The total extractable amount of copper in this end product was 541 mg kg^{-1} with water and calcium nitrate extractable copper accounting for 102.59 mg kg^{-1}. Composting of lignocellular materials derived from tanalised wood should therefore be exercised with caution.

500, 1000 and 1500 mg kg^{-1} Treatments

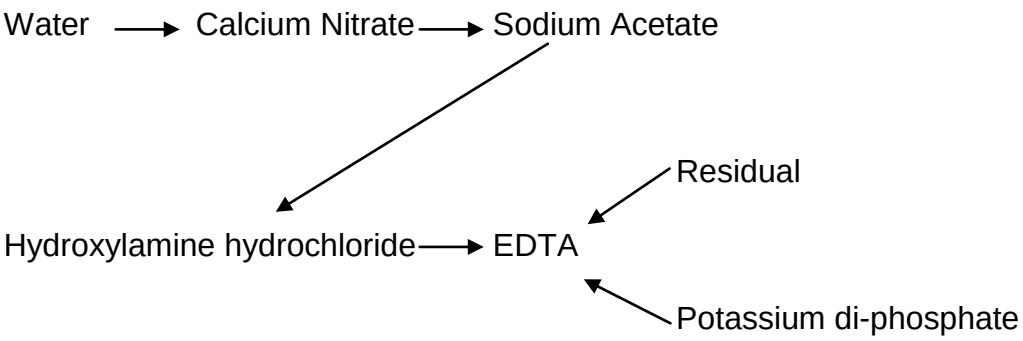
Copper was added to the material artificially as copper acetate and was seen to distribute itself very quickly amongst the various binding sites, as demonstrated by the elevated levels of copper in all the various fractions (except the residual) for the pots sampled at day 0. Ajmal *et. al.* (1998) demonstrated that maximum adsorption of copper applied as a solution of

$CuCl_2.2H_2O$ onto the surfaces of sawdust particles is reached within an hour of the material coming into contact with the solution. As there was a delay (unmeasured) between the preparation of the materials and their subsequent storage prior to analysis this may go some way to explaining the observed phenomenon. The cation exchange capacity of even the 1500 mg kg^{-1} treatment would also not have been saturated. An application of 1500 mg kg^{-1} would have an equivalency of only 4.7meq/100g fresh material. Cation exchange capacities have been found by other workers to increase over the composting period as humification proceeds and capacities of mature composts have been reported as varying from 36.9 to 228.6 meq/100g (Estrada et al 1987) and 23 to 138 $cmol_c/kg$ (Jokova *et al.* 1997). Unfortunately, cation exchange capacities of the samples have not been determined.

All the copper acetate treated materials behaved in a similar manner, the only real differences being the amounts of copper involved. Copper in the residual fraction was, like the 0 mg kg^{-1} treatment, released during composting to bind with ligands which were subsequently extractable with EDTA. The rate of decrease in residual forms of copper, as a percentage share of the total amount present in the material, was reduced with increasing treatment levels because most of the additional copper occupied binding sites which were extractable during the earlier stages of the scheme. Thus, these more extractable forms of copper occupied a greater proportion of the total amount present as the treatment levels increased. This was supported by the fact that the amounts of copper in the residual digestions for these three treatments

were not significantly different from each other, even up to the end of the experiment, although it was also possibly due to a lack of replicate samples.

High within-sample variability meant that it was not possible to draw firm conclusions about the trends seen. However, the data does indicate that, during composting of these types of materials, most of the copper in the 0 and 500 mg kg^{-1} treatments moves between the various extractable forms in the following manner:



However, decreases in the hydroxylamine hydrochloride extractable copper during the final phase of the study, as evidenced in the 1000 and 1500 mg kg^{-1} treatments, seemed to correspond better with increases in copper in the calcium nitrate extract and water extracts. Also, given that the percentage share of copper for the sodium acetate through to the residual fractions appears to reach equilibrium by the end of the experiment, it is likely that for these two treatments, ligands whose attached metals are extractable by water and calcium nitrate receive the excess copper. The above flow diagram is therefore by no means conclusive for all levels of copper treatment.

Reports in the literature of decreases in residual copper have not been found. On the contrary, reports of increases are the norm (Greenway and Song, 2002b; Zorpas *et. al.* 2000; Planquart *et. al.* 1999). Easily extractable copper is also normally reported as decreasing over time during composting (Szymanski *et. al.* 2005; Amir *et. al.* 2005; Paré *et. al.* 1999) although there are instances of increases attributed to leaching (Zorpas *et. al.* 2000 and 2003). In addition to this it was also seen that as there were no significant differences in the total extractable amount of copper in any of the treatments at any of the sampling times there was therefore no evidence of a concentrating effect of the composting processes on the total extractable or total digestible amounts of copper. Measurable changes in copper distribution during the experiment, as well as clear visible changes in the materials themselves, must be attributable to the ready decomposition of the grass. Moreover, sawdust, owing to its very high C:N ratio may have decomposed so slowly so that there was no observable overall concentrating of the copper levels by the composting process. The already high copper content of the sawdust would no doubt have hindered its breakdown further. The C:N ratio of the starting materials was already high, around 41:1

2.4 Conclusions

The use of this sequential extraction scheme has clearly shown that changes in operational copper speciation have occurred. Changes in phytoavailability, though not measured, are therefore likely. These changes would not have

been detected on the basis of a measurement of total copper alone; especially as the sum total of copper in all the fractions (total extractable copper) established that there were no changes in the overall amount of copper present in the treated materials. This work is therefore in agreement with claims asserted by other workers (e.g. Tisdell and Breslin, 1995) that knowledge of total content (of a metal) does not allow for a prediction of its bioavailability. However, a lack of sample replication for treatment and sampling dates meant that observed trends were not proven and only measurements of within-sample variability could be obtained.

Chapter 3.

Introduction

Building on the results of the previous investigation, Experiment 2 sought to establish the changes taking place in the partitioning of copper during the composting of a wider variety of waste materials. This time only two levels of copper were used (0 and 1000 mg kg^{-1}) to supplement the starting materials.

3.1 Compost Production: Materials and Methods

3.1.1 Compost production

Three different blends were prepared from the following four different waste materials:

1. Grass clippings,

These were obtained from a local croquet lawn. The lawn was mown in May 1999. Any large foreign materials were immediately removed and the clippings were then kept bagged and refrigerated until required.

2. Pig slurry

Collected from beneath the deck of a Shropshire pig unit housing pigs between 5 and 16 weeks old, fed on pellets containing 175 mg kg^{-1} copper.

3. Sewage sludge cake

Obtained from Severn Trent Water at their sewage treatment works in Coalport, Shropshire. During processing, waste solids were pressed causing the material to dewater and form slabs of ‘cake’. In preparation for composting these slabs were broken up, passed through a 12.6mm test sieve (Endecotts, UK) and the sieved cake stored at room temperature until required.

4. Pine sawdust

This was a fresh product produced from untreated timber and normally sold as guinea pig bedding.

These waste materials were characterised for moisture, nitrogen (Kjeldahl) and total copper (by digestion of oven dried material in aqua regia), the results of which are found in Table 3.1. The grass clippings, pig slurry and sewage sludge cake were all combined with the fresh sawdust to give a final C:N ratio of around 41-42:1 for all blends. Although this is rather high for composting - optimum ratios are usually 25-35:1 (FAO, 1987) - the slightly larger quantities of sawdust were felt to be a necessary compromise in order to be able to evenly apply aqueous copper treatments whilst still maintaining a sensible a sensible moisture content. This was of particular concern with the pig slurry and sawdust blend as the slurry was found to have a moisture content of 90%

Table 3.1 Characteristics of Compost Starting Materials

Waste Material	Moisture Content (%)	Total Nitrogen by Kjeldahl on a Fresh Basis (%)	Total Nitrogen by Kjeldahl on a Dry Matter Basis (%)	Total Copper on a Fresh Basis ($mgkg^{-1}$) by Aqua Regia Digestion	Total Copper on a Dry Matter Basis ($mgkg^{-1}$) by Aqua Regia Digestion
Grass Clippings	67.30	0.89	2.734709	4.64	14.22
Pig Slurry	90.32	0.52	5.35567	185.11	1912.38
Sewage Sludge Cake	76.47	1.26	5.346809	189.67	806.07
Sawdust	28.39	N/A*	N/A*	1.66	2.32

*N/A = Not available. The analysis could not be performed on this material due to excessive foaming as a result of the high carbon content.

and a Kjeldahl nitrogen content of 0.5%. All blends were treated with copper by addition of aqueous solutions of copper as copper acetate (Analar grade, Merck). Extra grass and sawdust blended material was prepared and treated with solutions of either copper sulphate (Analar grade, Merck) or cupric disodium salt of EDTA (15.0-16.5% Cu, Sigma).

In order to apply the copper solutions, prepared blends were spread out on plastic sheeting to a depth of 20mm and the solutions were carefully applied using dedicated watering cans. Materials were thoroughly mixed and the moisture content of each blend adjusted where necessary. Materials were then weighed, in triplicate, into individual composting units (3.75l polypropylene boxes 180x250x100 mm, by Sealfresh™) and the lids sealed with laboratory film. Units were placed in randomised blocks in three

incubators containing 8 units per block. Ratios of the various materials, weights of the contents of each unit and the treatments each blend received are given in Table 3.2.

The temperatures of the incubators were then adjusted over the course of the experiment to mimic those of a full-scale windrow:

* 45^{o}C for 4 days
* 50^{o}C for 3 days
* 55^{o}C for 1 week
* 60^{o}C for 6 weeks
* 55^{o}C for 29 weeks (curing)
* 25ºC for 8 weeks, 3 days.

Each composting unit was aerated on a weekly basis for the first 15 weeks by emptying the contents of each unit into a metal riffle box (360 x156 x 228 mm) and raking along the length of it six times with a hand held garden fork. The material was then returned to its box, resealed and returned to its original place in the incubator. After the 15th week the material was left to cure with only occasional turning. Water was added on an *ad hoc* basis as the material dried out.

A portion of each prepared blend was set aside for analysis and each unit was then sampled once at 15 weeks (105 days) and again at 45 weeks, 3 days (318 days). Samples were frozen at -20ºC until they could be analysed.

Table 3.2 Compost Blend Parameters and Characteristics

Blend Prepared	Ratio Waste:sawdust (m/m)	Initial %Moisture Content of Blend	Initial % Dry Matter Content of Blend	Estimated C:N ratio	Weight of Blend per Composting Unit (g)	Treatment Abbreviation	Treatment/s (mg Cu per kg of blend on fresh weight basis)	Treatment/s (mg Cu per kg of blend calculated on dry weight basis)	Initial % Volatile Solids by Loss on Ignition (± 95% CI)
Grass and sawdust	2.1 : 1	46.5	55.5	42:1	500	UGS AGS SGS EGS	0 1000 Cu-acetate 1000 Cu-sulphate 1000 Cu-EDTA	0 1871 Cu-acetate 1871 Cu-sulphate 1871 Cu-EDTA	95.4 (1.56) 95.1 (2.00) 96.1 (1.20) 95.5 (3.01)
Pig slurry and sawdust	2.2 : 1	61.4	38.6	42:1	1500	UPS APS	0 1000 Cu-acetate	0 2591 Cu-acetate	93.3 (0.94) 92.9 (0.72)
Sewage sludge cake and sawdust	0.87 : 1	50.7	49.3	41:1	800	UCS ACS	0 1000 Cu-acetate	0 2028 Cu-acetate	90.8 (7.51) 94.5 (6.15)

The grass clippings and sawdust are hereafter referred to as green waste blends and the other two – the pig slurry and sawdust and the sewage sludge cake and sawdust blends – are collectively referred to as brown waste blends.

3.2. Analyses

All materials for analysis were oven dried at 100ºC overnight and ground to pass a 1mm sieve (Cyclotec mill, Tecator, Sweden).

A comprehensive account of the analyses carried out, results obtained and relevant discussions are given in sections 3.2 through to 3.6. Briefly though, each sample was analysed for: copper by sequential extraction, total copper, total carbon, total sulphur, total nitrogen, functional groups by FTIR analysis and copper by chelating resin membrane using a diffusion sensitive approach. Water extracts were also obtained for samples taken at the start and the end of the experiment on which measurements of pH, EC, dissolved organic carbon (DOC), ammonium, nitrate, and phytotoxicity (cress seed germination) were made.

3.2.1 Sequential Extraction Analysis – Materials and Methods

A: BCR Extraction

As the Community Bureau of Reference (BCR) had developed a standardised 3-step sequential extraction scheme to facilitate the comparison of results

within the literature (Ure, *et. al.*, 1993) the initial decision taken was to use this scheme, rather than the seven-step scheme employed in the first experiment and to modify the method in two ways. Firstly, 2g of material were used rather than the 1g stipulated in the original BCR protocol (in a bid to improve precision) and secondly, a final strong acid digestion using *aqua regia* was included to determine residual copper. Details of this modified scheme are given in Table 3.3.

However, upon execution of the procedure it became readily apparent that the length of time involved was very similar to that required by the scheme of Tessier *et al.* (1979), and yet fewer data were generated. A number of samples were also lost during stage 3 due to their very vigorous reaction with the hydrogen peroxide. In view of these obvious drawbacks it was decided not to continue using this scheme on the later samples but rather to use the scheme of Tessier *et al.* (1979) and start again. However, the results obtained for the t=0 days samples obtained are included here for comparative purposes. Further details of the procedures are given in Appendix IV.

Subsequent to this, a selection of the samples – one sample chosen at random from one of the three blocks of each blend, treatment and sampling time - were reanalysed by sequential extraction using the Tessier *et. al.* (1979) scheme. Copper in each extract was then determined first by flame atomic absorption using only matrix-matched standards and then by flame atomic absorption using matrix-matched standards whilst employing standard

Table 3.3 BCR Extraction Scheme (Ure *et. al.* 1993) with Additional Residual Digestion

Stage	Fraction	Reagent	Shaking time and termperature
1	Extractable	40ml 0.11M Acetic acid	16 hours, room temperature
2	Easily Reducible	40ml 0.1M Hydroxylammonium chloride (acidified to pH 2.0 with c.HNO_3)	16 hours, room temperature
3	Organic	10 ml 8.8M H_2O_2	1 hour, covered at room temperature with occasional manual shaking; 1 hour covered with watch glass at 85^oC; Uncovered at 85^oC to reduce to a few ml.
		10 ml 8.8M H_2O_2	1 hour covered with watch glass at 85^oC; Uncovered at 85^oC to reduce to a few ml. Cool.
		50ml 1M Ammonium acetate (acidified to pH 2 with c.HNO_3)	16 hours, room temperature
4	Residual	30ml *aqua regia*	Transfer to 500ml Quickfit conical flask, dry at 100^oC, then heat gently on hotplate for 3 hours under reflux using cold-finger condensers. Cool, then filter via Whatman 541 filter paper and dilute to 100ml with deionised water.

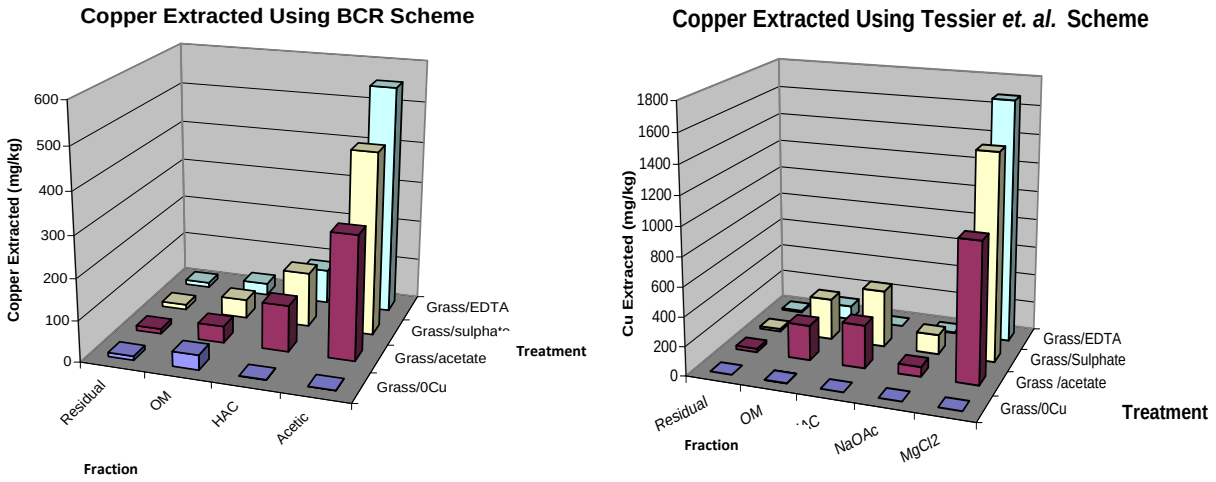
additions. The use of standard additions seeks to eliminate interferences in the air-acetylene flame which arise as a result of the presence of various unspecified compounds which are extracted from the sample at the same time as the analyte of interest. The standard additions technique was not used during the original set of analyses so the reanalysis of a selection of the samples was carried out in the manner described to check for flame interferences which may have originally occurred. Significant differences between the two approaches would indicate problems with flame interferences. Calculations for converting raw AAS data to levels of extracted copper are outlined in detail in Appendix II.

3.2.2 Comparison of the BCR Extraction Method With the Tessier *et. al.* Extraction Method for Samples Taken at t=0 Days

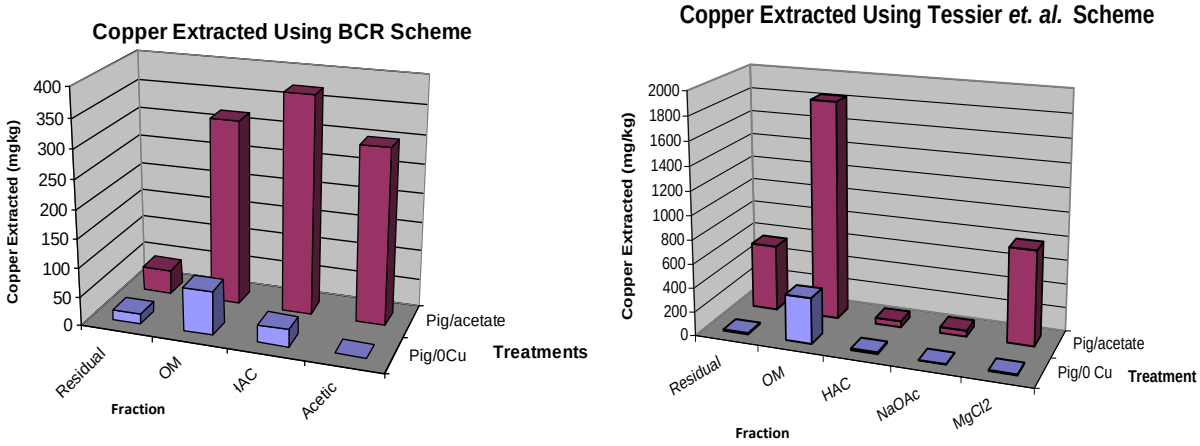
3.2.2.1 Results

As the BCR extraction method was only applied to the initial starting materials then results from these extractions are only compared with the results from the Tessier *et. al.* (1970) extractions performed on the same samples.

Data for all extractions are presented in both mg kg^{-1} and as a percentage of the total amount of copper extracted from each sample (the percentage share) in Figures 3.1 and 3.2 and also Tables 3.4 through to 3.9. Data were analysed fraction by fraction for each sample type by Analysis of Variance (ANOVA).



A Grass and Sawdust Copper Treated Blends



B. Pig Slurry and Sawdust Copper Treated Blends

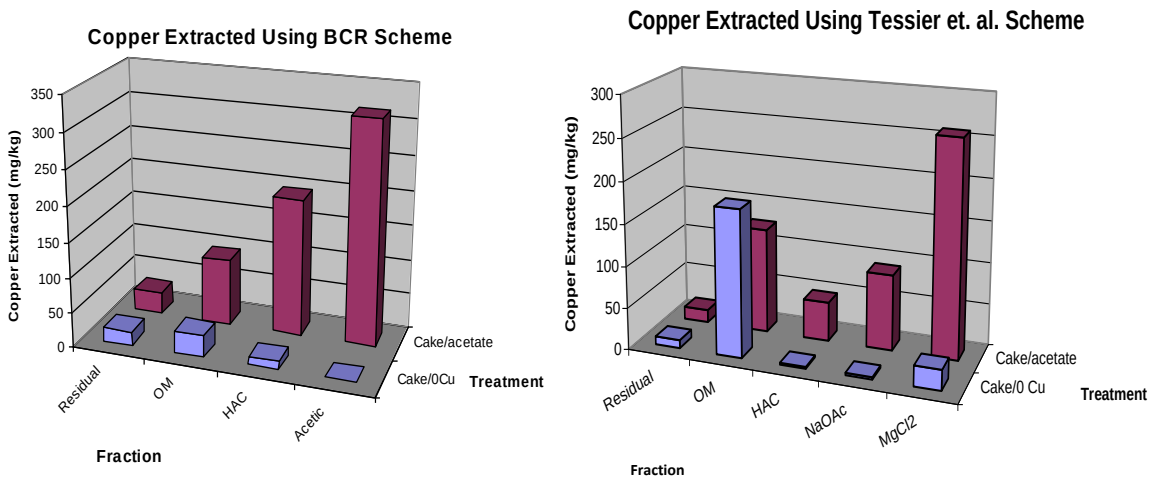
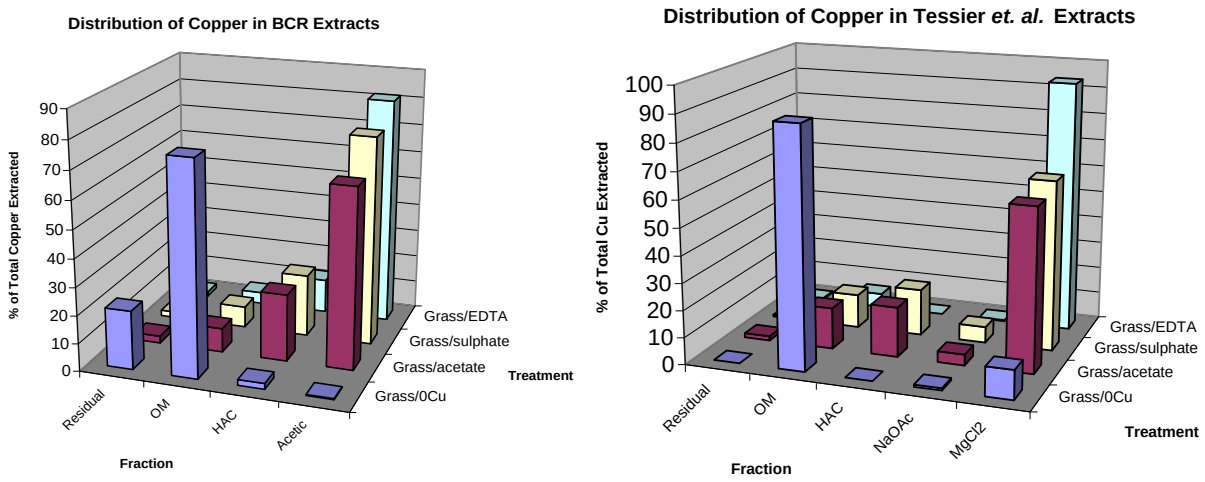
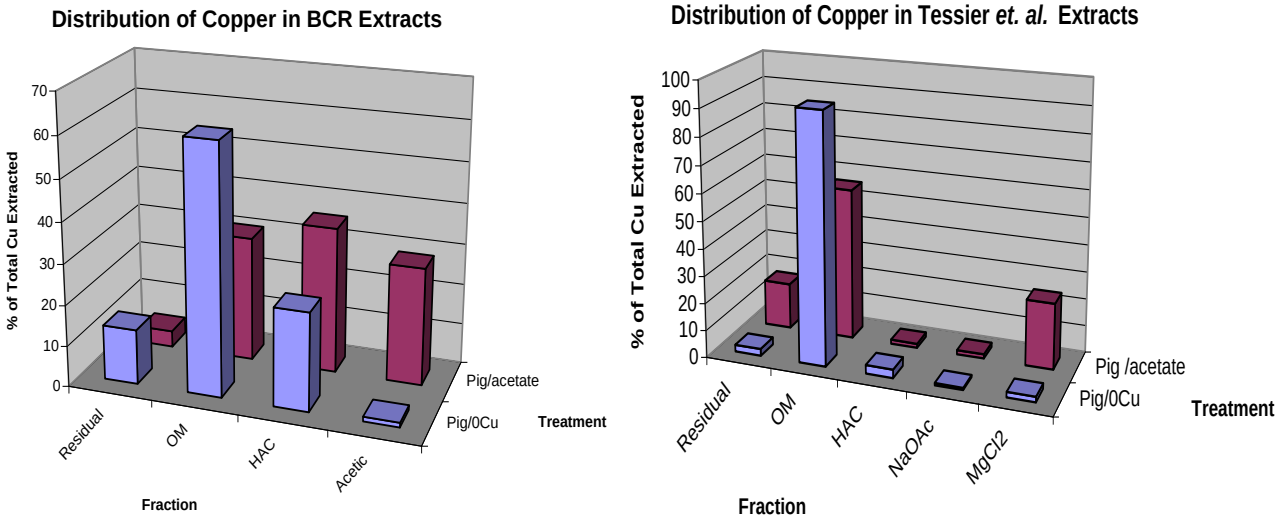


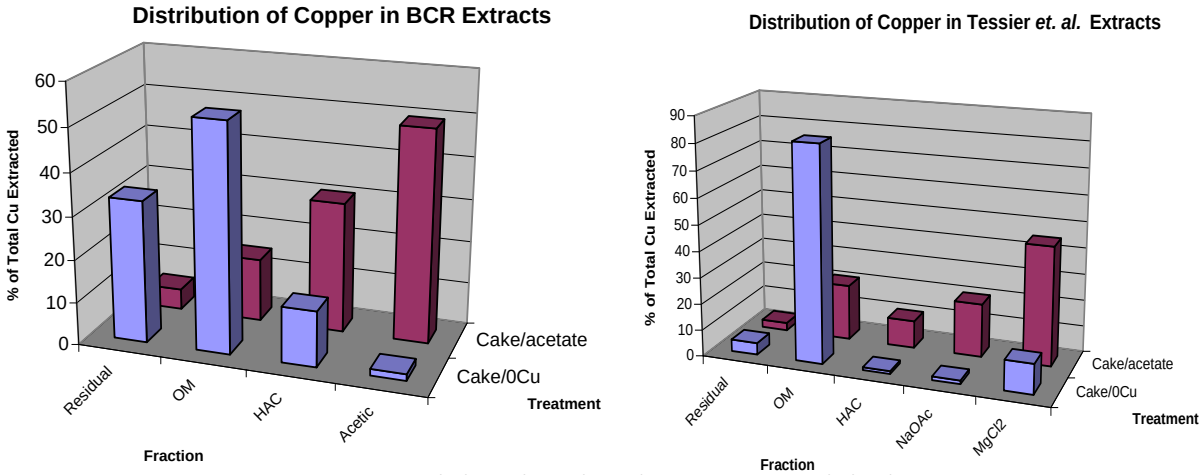
Fig 3.1 Comparison of the Amounts of Copper Extracted ($mgkg^{-1}$) from Initial Starting Materials by BCR and Tessier *et. al.* Sequential Extraction Schemes.



A Grass and Sawdust Copper Treated Blends



B. Pig Slurry and Sawdust Copper Treated Blends



C. Sewage Sludge Cake and Sawdust Copper Treated Blends

Fig 3.2 Comparison of the Distribution Within the Initial Starting Materials by BCR and Tessier *et. al.* Extraction Schemes.

Table 3.4 Comparison of the Amounts of Copper Extracted from the Grass and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results in mg kg^{-1}.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Total Extracted	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UGS	0.2^{a}	0.0^{a}	0.9^{a}	0.0^{a}	$35.12^{a,b}$	1.26^{a}	10.85^{a}	0.00	$49.8^{a,b}$	1.3^{a}
AGS	$299.6^{a,\ b}$	1020.9	98.2^{b}	294.7	40.45^{b}	239.98	14.26^{a}	24.42^{b}	$452.5^{b,c}$	1580.0^{d}
SGS	$442.6^{b,}$	1557.3^{c}	114.7^{b}	392.3	47.81^{b}	283.74	12.64^{a}	$17.83^{a,b}$	617.8^{c}	2251.2
EGS	$554.7^{b,}$	1688.3^{c}	$71.9^{a,b}$	0.0^{a}	$29.06^{a,b}$	95.49	11.77^{a}	10.72^{a}	667.5^{c}	1794.5^{d}

* Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated.

Table 3.5 Comparison of the Copper Extracted from the Grass and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results Given as Percentages of the Sum Total of Copper Extracted. Identical superscript lettering within each extracted fraction signify no significant differences between pairs of results.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UGS	0.402^{a}	0^{a}	2.40^{a}	0.00^{a}	75.95	100	21.25	0.00^{a}
AGS	64.33^{b}	64.49^{b}	24.04^{d}	$18.58^{b,c}$	8.55^{b}	15.33^{c}	3.09^{c}	1.60^{b}
SGS	70.26^{b}	69.18^{b}	$20.38^{c,d}$	$17.41^{b,c}$	$7.40^{a,b}$	12.62^{c}	$1.97^{b,c}$	$0.80^{a,b}$
EGS	79.12	94.08	14.25^{b}	0.00^{a}	4.74^{a}	$5.32^{a,b}$	$1.89^{b,c,}$	$0.60^{a,b}$

* Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated.

Table 3.6 Comparison of the Amounts of Copper Extracted from the Pig Slurry and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results in mg kg^{-1}.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Total Extracted	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UPS	1.1^{a}	12.4^{a}	$29.4^{a,b}$	13.2^{a}	75.2	381.8^{a}	17.2^{a}	10.6^{a}	123.0	418.0
APS	300.7	841.2	373.7	49.9^{b}	333.1^{a}	1831.0	46.1^{a}	555.0	1054.0	3277

* Key: UPS = untreated; APS = copper acetate treated

Table 3.7 Comparison of the Copper Extracted from the Pig Slurry and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results Given as Percentages of the Sum Total of Copper Extracted. Identical superscript lettering within each extracted fraction signify no significant differences between pairs of results.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UPS	$9.10^{a,b}$	2.98^{a}	24.91	3.17^{a}	$52.66^{a,b}$	91.31	13.33^{b}	2.54^{a}
APS	28.98^{b}	$25.64^{a,b}$	35.74	1.52^{a}	31.06^{a}	55.91^{b}	4.21^{a}	16.92^{b}

* Key: UPS = untreated; APS = copper acetate treated

Table 3.8 Comparison of the Amounts of Copper Extracted from the Sewage Sludge Cake and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results in mg kg^{-1}.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Total Extracted	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UCS	0.9[a]	28.0[a]	7.70[a]	2.24[a]	31.3	175.7	19.56[b]	9.6[a]	59.5	215.5
ACS	317.0[b]	357.9[b]	195.45	58.26	94.7[a]	119.7[a]	31.48	16.46[a,b]	638.7	552.3

*Key: UCS = untreated; ACS = copper acetate treated

Table 3.9 Comparison of the Copper Extracted from the Sewage Sludge Cake and Sawdust Blends by Both BCR and Tessier Sequential Extraction Schemes. Results Given as Percentages of the Sum Total of Copper Extracted. Identical superscript lettering within each extracted fraction signify no significant differences between pairs of results.

Treatment*	Fraction 1		Fraction 2		Fraction 3		Fraction 4	
	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme	BCR Scheme	Tessier *et al* Scheme
UCS	15.03[a]	13.00[a]	13.05[a,b]	1.06[a]	43.82[a]	81.44	28.11	4.50[a]
ACS	41.50[a,b]	64.65[b]	25.64[b]	10.59[a]	20.47[a]	21.78[a]	12.39[a]	2.99[a]

*Key: UCS = untreated; ACS = copper acetate treated

Where two or more interactions were being analysed using Tukey's method of multiple comparisons between means at the 95% confidence interval was used.

The two schemes have an unequal number of extractions. So, to compare them effectively, fraction one of the BCR scheme (the acetic acid extraction) was compared with the sum of the first two fractions in the Tessier scheme (the magnesium chloride and sodium acetate extractions) as it was assumed that these would be reasonably comparable. Others have also taken this approach (Usero *et al.,* 1998).

The software program Genstat® (8th Edition) was used for all statistical analyses. A sample Analysis of Variance table is given in Appendix IV.

A: All Grass/Sawdust Blends

a) Fraction 1 – Easily Extractable Copper

In terms of the amounts of copper extracted during the first extraction, all factors (extraction scheme and treatment type) and interactions (extraction scheme x treatment type) were very highly significant ($p \leq 0.001$). Overall, the Tessier scheme was found to extract significantly more copper than the BCR scheme from those materials which had been treated with additional copper. No significant differences though were found between the extraction schemes for the control (grass/sawdust blend with no added copper). Copper extracted

from the UGS by both the BCR and Tessier schemes was significantly less than that extracted from all the other copper treated materials.

Analysis of the percentage share data found that extraction scheme effects were highly significant ($p=0.002$) and that treatment type and extraction scheme x treatment interactions were both very highly significant ($p \leq 0.001$). As with the data for the amounts of copper extracted, no significant differences were found between the extraction schemes for the control. Similarly, the proportion of copper extracted from the UGS by both schemes was significantly smaller than that extracted from all the other copper treated materials. The proportions of extractable copper in the AGS and SGS materials did not differ significantly from each other and also did not depend on the extraction scheme used. Only the EGS differed. This contained a significantly greater proportion of extractable copper compared with the other treatments and the results did depend on the extraction scheme used, with the Tessier scheme extracting a greater proportion of copper than the BCR scheme.

b) Fraction 2 – Reducible Fraction

All factors and interactions for the amounts of copper extracted were very highly significant ($p \leq 0.001$) and, as for Fraction 1, each treatment type (except the control) gave results which depended on the extraction scheme used. In the AGS and SGS materials the Tessier scheme extracted significantly more copper than the BCR scheme (98.2 mg kg^{-1} by the BCR scheme compared with 294.7 mg kg^{-1} by the Tessier scheme). This is surprising given that the

Tessier scheme also extracted more copper than the BCR scheme in Fraction 1. However, the Tessier scheme failed to extract any detectable copper in the material and consequently this treatment did not differ significantly from the control. The BCR scheme extracted a detectable amount of copper from the EGS but, as with the Tessier scheme, this also did not differ significantly from the control.

All factors and interactions for the percentage share data for this fraction were found to be very highly significant ($p \leq 0.001$). Although at this stage the Tessier procedure extracted significantly larger amounts of copper than the BCR, when considered in terms of the percentage share of extractable copper then quite the reverse was seen. Averaged out across all the treatments the hydroxlyamine hydrochloride extraction in the Tessier scheme removed a much smaller proportion of the total extractable amount than the same stage in the BCR scheme.

c) Fraction 3 – Oxidisable Fraction

For the amounts of copper extracted, all factors and interactions were very highly significant ($p \leq 0.001$). The Tessier scheme extracted significantly more copper from all the copper amended material than the BCR scheme and further significant differences were seen between all treatments where the Tessier scheme was used. No significant differences though were seen between the two schemes for the control materials and no significant differences were seen between the various treatments where the BCR scheme was used. As with the results for the amounts of copper extracted,

analysis of the percentage share data demonstrated that all sources of variation were very highly significant ($p \leq 0.001$). The control also contained a significantly greater percentage of copper in the 'oxidisable' fraction than all the other treatments with the BCR scheme extracting 75.95% of the total extractable copper and the Tessier scheme extracting 100.00% of the total extractable amount. Both schemes were significantly different from each other in this case. Where copper was added as either copper acetate or sulphate than the Tessier scheme extracted a significantly greater proportion of the total extractable copper (15.33% and 12.62% respectively) than the BCR scheme (8.55% and 7.4%). There was no significant difference though between the two schemes for the EGS although the results in both cases were significantly lower than for nearly all the other treatments.

d) Fraction 4 – Residual Copper

Amounts of copper measured in this final, residual fraction were not found to be significantly related to the extraction scheme used ($p=0.535$). However, all other sources of variation were very highly significant ($p \leq 0.001$). With the BCR scheme, none of the treatments, including the control, were significantly different from each other, nor were they different from the SGS and EGS materials which extracted by the Tessier scheme. Conversely, with the Tessier scheme, significant differences were seen between the control and all other treatments (extracted by both schemes) and between the EGS and all the other treatments. No difference though was seen between the AGS and SGS.

All interactions for the percentage share data were very highly significant ($p \leq 0.001$). Significantly greater proportions of copper were extracted using the BCR scheme than with the Tessier scheme for the control and the AGS. No significant differences were found between the SGS and EGS treatments for either scheme. The largest proportion of residual copper was extracted from the control by the BCR scheme (21.25% of the total extractable copper) which was in sharp contrast to the Tessier scheme (0% of the total extractable copper).

e) Total Extractable Copper – Sum Total of all Fractions (mg kg^{-1})

All factors and interactions were found to be very highly significant ($p \leq 0.001$). No significant differences were observed between the two schemes when they were used to extract copper from the control material. However, for the amended materials, the Tessier scheme was found to extract significantly larger amounts of copper than the BCR scheme. However, more variation between treatments was seen with the Tessier scheme as more treatments differed significantly from each other. Only the AGS and EGS treatments were not significantly different from each other. With the BCR scheme, no differences were found between the AGS, SGS and EGS treatments. The control was found to contain significantly less copper than all the SGS and EGS treatments, but not the AGS.

B: Pig Slurry/Sawdust Blends

a) Fraction 1 – Easily Extractable Copper

For the amounts of copper extracted, all effects and interactions were found to be very highly significant. Copper extractable from the control samples was not found to depend on the extraction scheme chosen. The BCR scheme extracted 1.4 mg kg^{-1} of copper and the Tessier scheme extracted 12.4 mg kg^{-1}. Nevertheless, these were not found to be significantly different from each other. Where copper was added as copper acetate, these samples contained, as expected, significantly more extractable copper than the control samples. However, the amounts extracted did depend on the extraction scheme chosen, with the Tessier scheme extracting significantly more copper (841.2 mg kg^{-1}) than the BCR scheme (300.7 mg kg^{-1}).

A very different pattern was seen in the percentage share data. Whilst treatment effects were found to be highly significant (p=0.005), neither extraction scheme effects nor extraction scheme x treatment interactions were found to be all significant (p=0.380 and 0.791 respectively). The average amount of copper extracted from the control by the two schemes was 6.04% of the total amount of extractable copper, whilst for the copper acetate amended samples, the average was 27.31%, more than four times that of the control. Least significant differences of the treatment means at the 5% level showed them to be significantly different (LSD = 12.30%). However, Tukey's method failed to find any significant differences between individual pairs.

b) Fraction 2 – Reducible Fraction

All effects and interactions were found to be very highly significant (p≤0.001) for the amount of copper extracted. No differences between extraction schemes were seen for copper extracted from the control samples. However, for the APS, the BCR scheme extracted more than seven times the amount of copper than the Tessier scheme. Indeed, the amount extracted by the Tessier scheme was so small in comparison (49.9 mg kg^{-1}), that this was not even found to be significantly different from the amount extracted from the control samples by the BCR scheme (29.4 mg kg^{-1}).

Extraction scheme effects were found to be highly significant and treatment effects and extraction scheme x treatment interactions were highly significant (p=0.007 and 0.002 respectively). As with the data of the amounts of copper extracted, the BCR scheme extracted a significantly greater proportion of extractable copper than the Tessier scheme (30.33% for the BCR scheme; 2.35% for the Tessier scheme as averages over the two treatments; LSD [5% level] = 2.84%). Tukey's multiple comparison analysis found that the BCR scheme extracted a significantly greater proportion of extractable copper from the copper amended materials than from the control, whilst the Tessier scheme did not show a significant difference between the proportion of copper extractable from the control and the proportion extractable from the APS.

c) Fraction 3 – Oxidisable Fraction

All effects and interactions for the amount of copper extractable at this stage were very highly significant (p≤0.001). As expected, the APS materials were found to contain significantly more extractable copper than the control (1082

mg kg^{-1} compared with 228 mg kg^{-1} when averaged across the extraction schemes; LSD [5% level] = 76.6) and the Tessier scheme extracted significantly more copper than the BCR scheme, (1106 mg kg^{-1} compared with 204 mg kg^{-1} when averaged across treatment levels; LSD [5% level] = 76.6). When individual comparisons of pairs were made, the control was found to contain significantly less than the APS regardless of the scheme used.

Extraction scheme x treatment interactions were not seen to be significant for the percentage share data ($p=0.211$), although extraction scheme effects were found to be very highly significant ($p\leq0.001$) and treatment effects highly significant ($p\leq0.001$). Material treated with the copper acetate appeared to contain a lower proportion of copper than the untreated material. However, this difference was only found to be significant where the Tessier scheme was used. No difference between the control and the copper treated material was established by using the BCR scheme. In addition to this, no significant difference was found between the control, as extracted by the BCR scheme and the copper treated material as extracted by the Tessier scheme.

d) Fraction 4 – Residual Copper

All effects and interactions were very highly significant ($p\leq0.001$) with the Tessier scheme extracting a much larger quantity of copper than the BCR scheme and the APS containing, on average, significantly more copper than the untreated (301 mg kg^{-1} for the Tessier scheme as compared with 14 mg kg^{-1} for the BCR scheme). The Tessier scheme extracted 555 mg kg^{-1} of copper from the APS and this was significantly different from both the control

material (both extraction schemes) and from the APS as extracted by the BCR scheme. No other comparisons were found to have significant differences between them.

Extraction scheme effects were not found to be significantly different for the percentage share data ($p=0.443$), nor were treatment effects ($p=0.066$). Extraction scheme x treatment interactions were very highly significant though ($p\leq0.001$). Averaged out across both treatments, the proportion of extractable copper was the same regardless of the scheme used (8.77% for the BCR scheme; 9.73% for the Tessier scheme; LSD [5%level] = 2.876). The same was also true across both the treatments, the APS treatment not containing a significantly greater proportion of extractable copper than the control (7.94% for the control; 10.57% for the copper acetate treatment; LSD [5%level] = 2.876).

e) Total Extractable Copper – Sum Total of all Fractions (mg kg^{-1})

All effects and interactions were found to be very highly significant ($p\leq0.001$). On average, the Tessier scheme extracted more copper from the materials than the BCR scheme (588 mg kg^{-1} for the BCR and 1848 mg kg^{-1} for the Tessier scheme; LSD [5% level] = 88.6 mg kg^{-1}). As expected, the copper acetate treated materials contained, on average, significantly more extractable copper than the untreated (271 mg kg^{-1} for the control and 2165 mg kg^{-1} for the APS). All comparisons of individual pairs of results showed significant differences.

C: Sewage Sludge Cake/Sawdust Blends

a) Fraction 1 – Easily Extractable Copper

Neither extraction scheme effects nor extraction scheme x treatment interactions were found to be significant. Treatment effects however were very highly significant ($p \leq 0.001$) with the ACS containing significantly greater quantities of extractable copper than the untreated. Nevertheless, over both treatments, neither of the two extraction schemes differed in the amounts of copper extracted from this material (159.0 mg kg^{-1} for the BCR scheme; 192.9 mg kg^{-1} for the Tessier scheme; LSD [5% level] = 35.46 mg kg^{-1}). No significant differences were found between extraction schemes for the control or the ACS.

In a similar way, treatment effects were found to be highly significant for the percentage share data ($p=0.005$), whilst extraction scheme effects and extraction scheme x treatment interactions were not significant at all. Averaged across both schemes the control contained a significantly smaller proportion of extractable copper (14.02%; LSD [5% level] = 21.78%) than the ACS (53.08% LSD [5% level] = 21.78%). However, comparisons of individual pairs of results using Tukey's 95% confidence intervals, found that the control material and the ACS were only significantly different from each other when extracted by the Tessier scheme. No significant differences between the two treatments were observed when the BCR scheme was used.

b) Fraction 2 – Reducible Fraction

All sources of variation for the quantities of copper extracted in fraction 2 were found to be very highly significant ($p \leq 0.001$). Overall, the BCR scheme was found to extract significantly larger amounts of copper than the Tessier scheme and the ACS materials were found to contain significantly larger amounts than the control. No significant differences were found between the extraction schemes used for the UCS although, for the ACS, the BCR scheme extracted significantly larger quantities of copper than did the Tessier scheme (195.45 mg kg^{-1} for the BCR scheme; 58.26 mg kg^{-1} for the Tessier scheme).

Extraction scheme and treatment effects were found to be highly significant ($p=0.003$ and 0.008 respectively) although no significant interactions between the two were found. As seen with the amounts of copper extracted, the BCR scheme was found to extract a greater proportion of the extractable copper (19.35%) than the Tessier scheme did (5.83%; LSD [5% level] = 6.97%). The ACS also contained a significantly greater proportion of extractable copper (18.12%) compared with the UCS (7.06%; LSD [5% level] = 6.97%), although no significant

differences were seen between the two treatments for individual schemes.

c) Fraction 3 – Oxidisable Fraction

Extraction scheme effects and extraction scheme x treatment interactions for the quantities of copper extracted at this stage were found to be very highly significant ($p \leq 0.001$). Treatment effects were not found to be significant. Overall, across both treatments, the Tessier scheme extracted significantly more copper from the oxidisable fraction than the BCR scheme (147.7 mg kg^{-1}

for the Tessier scheme; 63.0 mg kg^{-1} for the BCR scheme; LSD [5% level] = 21.54 mg kg^{-1}). As might be expected, the BCR scheme extracted significantly more copper from the ACS than from the control. However, the reverse was true for the Tessier scheme.

Extraction scheme effects and extraction scheme x treatment interactions were found to be significant for the percentage share data (p=0.010 and 0.013 respectively). Treatment effects which had not been significant for the amounts of copper extracted were now very highly significant ($p \leq 0.001$). Significant differences were seen between the UCS and the ACS treatment when the Tessier scheme had been used, the control containing significantly more copper in the oxidisable fraction. No significant differences were seen though when the BCR scheme was used.

d) Fraction 4 – Residual Copper

Both extraction scheme and treatment effects were very highly significant ($p \leq 0.001$). Interactions between these two effects were not found to be significant. The BCR scheme was found, on average, to extract significantly more copper than the Tessier scheme (25.52 mg kg^{-1} for the BCR scheme; 13.03 mg kg^{-1} for the Tessier scheme; LSD [5% level] = 3.727 mg kg^{-1}) and the ACS contained, on average, significantly more copper than the control (14.58 mg kg^{-1} for the control; 23.97 mg kg^{-1} for the copper amended treatment; LSD [5% level] = 3.727 mg kg^{-1}). Individual comparisons of pairs of results using Tukey's multiple comparisons method (95% confidence intervals) found no significant differences between the two treatments for

extraction by the Tessier scheme but a significant difference between them when the BCR scheme was used.

No effects or interactions were found to be significant for the percentage share data and Tukey's multiple comparison method found no significant differences between any pairs of results. This may be due to the large variation in the data - the coefficient of variation was 98.9%.

e) Total Extractable Copper – Sum Total of all Fractions (mg kg^{-1})

Extraction scheme effects were significant (p=0.047) and treatment effects and extraction scheme x treatment interactions were very highly significant (p≤0.001). Across both treatments, the Tessier scheme extracted significantly more copper than the BCR scheme (349.1 mg kg^{-1} for the BCR scheme; 383.9 mg kg^{-1} for the Tessier scheme; LSD [5% 34.09 mg kg^{-1}) although Tukey's multiple comparisons found that the extractive abilities of the Tessier scheme only exceeded those of the BCR for the control material. For the ACS, the BCR scheme was found to extract significantly larger amounts than the Tessier scheme. The copper amended treatment was found to contain significantly more copper than the untreated materials, regardless of the extraction scheme used.

3.2.2.2 Discussion

The work undertaken here to compare the two schemes in this composting project is unique as it is usually the case that when sequential extractions are carried out, only one type of scheme is chosen. Comparisons between the BCR and Tessier schemes have been made but so far their use has been limited to soils, sediments, sewage sludges and fly ash and findings are sometimes contradictory. Chwastowska and Skalmowski (1997) is the only example to date of a comparison between a BCR developed extraction procedure and the classical Tessier scheme where the two schemes have been applied to composted materials. However, the BCR procedure used was a single extraction designed originally for soils and not the three-step procedure recommended for sediment analysis. In total, no comparisons between the Tessier scheme and the three-step BCR scheme in compost studies have been reported.

In this study, metal distribution amongst the various fractions within the green waste samples followed very similar trends, even if the actual amounts extracted or the actual percentage share for individual fractions differed significantly. These findings are similar to those of van Hullebusch *et. al.* (2005) who, during their investigation of sewage sludges, found that the same two schemes, whilst showing similar trends in metal distribution were sufficiently repeatable and reproducible, differences between their performances existed.

In composting the grass/sawdust materials, the control material demonstrated the least variation of all the treatments, with no significant differences

occurring between the two schemes, other than in the oxidisable fraction. As there were no significant differences between the two schemes for the amounts of copper extracted within fraction 1 of the control material and also no differences between them for the proportion of extractable copper within this fraction for the control and the AGS and SGS materials, it was therefore concluded that the original assumption, that both the magnesium chloride and sodium acetate extractions of the Tessier scheme would be equivalent to the acetic acid extraction of the BCR scheme, was correct for these materials. Distribution of copper amongst the AGS and SGS materials was, both in terms of the amounts extracted and as a percentage share, in the order of Fraction 1 (exchangeable) > Fraction 2 (reducible) > Fraction 3 (oxidisable) > Fraction 4 (residual). This was true for both schemes and also for the EGS where the BCR scheme was used. The Tessier scheme was slightly different for this treatment in that no copper was extracted in the second fraction. This gave the slightly different distribution of Fraction 1 (exchangeable) > Fraction 3 (oxidisable) > Fraction 4 (reducible) > Fraction 2 (reducible). In general though, the trends in copper distribution among the various fractions were similar for both schemes. A number of other workers have also reported similarities between the BCR and Tessier schemes. In their analysis of contaminated sewage sludges Turek *et. al.* (2005) found that results obtained by the two schemes appeared to be consistent. Perez-Cid *et. al.* (1996), working on urban sludge samples, also found similar performances for both schemes and, furthermore, work on manganese distribution in fly ash (Kalembkiewicz and Sitarz-Palczak, 2005) revealed that the two schemes gave both comparable and reproducible results.

Overall, for the copper amended grass/sawdust materials, the Tessier scheme was found to be a far more efficient procedure than the BCR scheme, extracting more than 2-3 times the amount of copper from these materials. This is surprising as it might reasonably be expected that even if copper was not removed during the earlier stages of the BCR scheme, it would certainly have been extracted by the *aqua regia* digestion in Fraction 4. This final step did not extract more 14.26 mg kg^{-1} from any of the grass/sawdust blends. It might be the case that this was the maximum amount that could be removed by this volume of *aqua regia* from these materials during 3 hours of refluxing. Possibly a greater volume of *aqua regia* and/or longer digestion times would have recovered the remaining copper. Drying and then ashing the residue from stage 3 might also have worked, as might the method of determining residual copper used with the Tessier scheme. In contrast, van Hullebusch *et. al.* (2005) found that an overestimation was made by the BCR scheme of metals within the reducible fraction. This was attributed to the release of substantial amounts of elements bound to the organic/sulphide fraction and led the authors to conclude that use of the BCR scheme was of limited value when studying anaerobic matrices.

Greater differences between the two schemes were more evident when they were applied to the analysis of the brown waste blends. Whilst greater extraction efficiencies have been seen in the extraction of metals from the reducible fraction of the materials by the Tessier scheme and in the extraction of metals held within the oxidisable fraction by the BCR scheme in sewage

sludge samples (Albores *et. al.,* 2000) and soils (Rakasataya *et. al.*, 1996), quite the opposite was found here. In fraction 2, the BCR scheme exceeded the Tessier scheme's ability to extract copper from these materials. No significant differences were seen between the two schemes in the amounts of copper extracted from the control materials in this fraction. However, the proportion of copper extracted from the UPS was greater in the BCR scheme and significant differences were also seen in the copper acetate amended treatments for both brown waste blends, both in terms of the amounts and the proportions of copper extracted. This is possibly a reflection of the differences in extracting conditions for the two schemes. Both use an aqueous solution of hydroxylamine hydrochloride, but the BCR scheme uses twice the volume of reagent at more than double the concentration which, whilst not made up in 25% acetic acid and heated for 6 hours at 96°C, is acidified to pH 2.0 and extracted for a much longer period of time – 16 hours – although at room temperature. Clearly, the presence of 25% acetic acid in the Tessier *et. al.* scheme, made no difference here. This is in contrast to the findings of Albores *et. al.* (2000). In their research sewage sludge samples collected from an urban wastewater treatment plant in Spain were subjected to both sequential extraction schemes and the results compared with single extractions using identical operating conditions to those used in each of the BCR and Tessier fractions. Their aim was to see if either of the extraction schemes could be replaced with either one or more of the single extractions and yet still provide sufficient information on metal extractability. The two schemes were compared with the single extractions and not compared directly with each other. However, the information provided for each fraction (mean quantities of

metals extracted; standard deviations and numbers of replicates) was sufficient to be able to determine the presence or absence of significant differences. Results of this can be found in Appendix V. Albores *et. al.* also followed the example set by Usero *et al.,* (1998) in which the first BCR scheme fraction was compared with the sum of the first two Tessier fractions was followed. In almost all cases significant differences were found between the two methods with the Tessier scheme extracting the largest quantities of metals in almost all fractions, particularly the reducible fraction. The only notable exception to this was the oxidisable fraction for sample A in which the BCR extracted more metals than the Tessier scheme. The greater extraction efficiency of the Tessier scheme for the reducible fraction was ascribed to the presence of 25% acetic acid, and the ability of the BCR scheme to extract more metals from the oxidisable fraction in sample A attributed to the longer extraction times coupled with the complete evaporation of the reagents. The Tessier scheme was also more efficient overall in extracting copper from the sludges. The difference in the total amount of copper extracted by the two schemes was significant, though they were less marked than in the materials under investigation here. Similar relationships between the two schemes within the reducible and oxidisable fractions have been reported within synthetic model soils (Rakasataya *et. al.*, 1996) for the same reasons. Here, lead redistribution from spiked phases was found to be highest in the reducible fraction in the Tessier scheme and highest in the oxidisable fraction in the BCR scheme.

It is more likely then that, in the materials under investigation, the greater overall extraction efficiency of the BCR scheme during the reducible stage of the BCR scheme reflected the findings of van Hullebusch *et. al.* (2005). Here, the BCR scheme overestimated the amounts of copper held within the reducible phase by extracting organically bound copper that should, if the scheme had been adequately 'phase selective' (Miller *et. al.* 1986a), have been extracted in the oxidising fraction.

Large differences between the two schemes have also been found for metal distribution in contaminated Montana reference soils (Sutherland and Tack, 2003) with lead extracted into reducible fractions showing especially large differences. These results contrast with those found by Ho and Evans (1997) who, on analysing the same reference soils using the BCR scheme, found that results for copper and other metals were comparable with results found previously using the Tessier et al scheme. In fact, in the majority of cases, significant differences are usually found between the two schemes. Alvarez *et. al.* (2006) found that the results for manganese extraction in alkaline soils depended on the scheme used, with the BCR scheme extracting significantly more manganese in the reducible fraction than the Tessier scheme and with more manganese being extracted in the residual fraction by the Tessier scheme than by the BCR scheme. The residual fraction of the Tessier scheme has also been found to extract significantly more copper than the BCR scheme (Usero *et. al.* 1998). Polish workers (Kalembkiewicz and Soco, 2005) found that while the Tessier *et. al.* scheme reliably extracted chromium from soil and recovered 96.5% of the total present, the BCR scheme did not,

recovering only 18.2%. Lopez-Sanchez *et. al.* (1993) saw marked differences in heavy metal distribution within fractions of four sediment samples. In these samples the BCR scheme only extracted metals at step three, the oxidising step whilst with the Tessier scheme all non-residual metals occurred in the second, third and fourth steps (carbonate bound, reducible and oxidisable).

For all brown waste treatments, other than the ACS, the Tessier scheme far exceeded the BCR scheme in terms of its ability to extract copper during the oxidising stage. For the pig slurry/sawdust blends the difference between the two schemes, in terms of the proportion of copper extractable, could be accounted for by the additional copper available for extraction which was not initially extracted during the reducible stage in the Tessier scheme. However, this explanation does not adequately explain why the Tessier scheme extracted much larger quantities of copper from the oxidisable fraction of these materials, neither does it explain the significant differences seen between the two procedures in the UCS blends. The BCR scheme employed larger volumes of hydrogen peroxide and longer heating times and would have been expected to extract more of the metal, as was found by Albores *et. al.,* (2000) and Rakasataya *et. al.*, (1996). This did not appear to happen and more copper was extracted during the residual step. Copper already present in the sewage sludge cake/sawdust blend must therefore be very tightly bound within the organic complexes of the sewage sludge cake. No significant differences were found between the two schemes for the ACS, indicating that any additional organic-metals complexes formed as a result of the copper treatment were readily extractable at this stage in both schemes. The use of

standard additions was not employed during the analysis of the extracts by flame atomic absorption spectophotometry so it is possible that the extraction of various organic complexes and molecules may have caused interferences within the flame with either of the schemes and affected the results. This would be despite the fact that others have noted that fewer matrix effects have seen with the BCR scheme (Dutta *et. al.* 2005 and Perez-Cid *et. al.* 1996).

It is clear that much further work would need to be carried out to draw any firm conclusions as to the difference in behaviour of the two schemes in these materials. No investigations of this kind have so far been reported. Only in the analysis of soils, sediments, sludges and ash have the differences between the two schemes been studied in detail. It is therefore somewhat difficult to assess the reasons for the differing behaviour of the schemes in the analysis of, what are very different, organic materials. Any conclusions made at this stage are therefore, by necessity, speculative.

3.2.2.3 Summary of Findings

The main findings of this comparison between the two extraction schemes are as follows:

- This is the first time that such a comparison of the two schemes has been made on composted materials

- It is reasonable to treat the magnesium chloride and sodium acetate fractions of the Tessier scheme as being approximately equivalent to the first fraction (acetic acid extraction) of the BCR scheme.
- Both schemes showed similar trends in metal distribution within the various treatments of the grass/sawdust based materials, although amounts and proportions differed significantly.
- The Tessier scheme proved to be more efficient than the BCR scheme at extracting copper from the copper amended green waste blends.
- Fewer differences were found between the two schemes for the green waste control material, than for the copper amended green waste materials.
- For all brown waste treatments, other than the UCS, the BCR scheme was more efficient than the Tessier scheme at extracting copper during the reducible phase, possibly owing to removal of copper from the oxidisable phases thus highlighting the possibility of problems with lack of phase selectivity.
- For all brown waste treatments, other than the ACS blend, the Tessier scheme far exceeded the BCR scheme in terms of its ability to extract copper during the oxidising stage.

3.2.3 Sequential Extraction Analysis of Samples Taken at t=0, 105 and 318 Days using the Method of Tessier *et. al.* (1979)

3.2.3.1 Results and Discussion of the Reanalysis of a Selection of Samples Using Standard Additions

Data were analysed fraction by fraction for each sampling date by Analysis of Variance using Tukey's method of multiple comparisons between means at the 95% confidence interval. Results for the four treatments of the grass/sawdust blend are summarised in Tables 3.10 to 3.12. Tables 3.13 to 3.15 cover data for the unamended and the copper acetate treatments of the grass/sawdust, pig slurry/sawdust and sewage sludge cake/sawdust blends. As the purpose of the exercise was to determine to what extent the use of standard additions made a difference to the amount of copper determined in the extract itself results are given in mgl^{-1} of the extractant. Further calculations to ascertain the total amount extracted from the sample (in mg Cu per kg material) were unnecessary.

There were very few problems with flame interferences as, in the majority of cases, the use of standard additions made very little difference to the results obtained. Analysis of the data revealed that method effects (use of matrix-matched standards only or matrix-matched standards plus standard additions) were rarely significant. Even where method effects were found to be significant e.g. in the residual fraction for grass/sawdust samples taken at t = 0 days ($p=0.015$), it was often the case that Tukey's

Table 3.10 Comparison of the Amounts of Copper Extracted from Grass and Sawdust Blend Samples Taken at t= 0 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.13^{a}	0.11^{a}	0.03^{a}	0.02^{a}
AGS	66.72^{b}	64.59^{b}	15.14^{c}	15.47^{c}	15.61^{b}	16.31^{b}	13.51^{c}	13.14^{c}	0.95^{d}	$0.84^{c,d}$
SGS	104.08^{c}	97.76^{c}	18.52^{d}	19.48^{d}	17.16^{b}	16.67^{b}	14.26^{c}	13.70^{c}	$0.83^{c,d}$	0.73^{c}
EGS	218.60	237.38	11.98^{b}	12.47^{b}	1.00^{a}	0.98^{a}	3.15^{b}	3.13^{b}	0.27^{b}	0.24^{b}

Table 3.11 Comparison of the Amounts of Copper Extracted from Grass and Sawdust Blend Samples Taken at t= 105 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.07^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.03^{a}	0.02^{a}	0.67^{a}	0.64^{a}	0.08^{a}	0.07^{a}
AGS	27.85^{b}	27.00^{b}	5.92^{b}	5.98^{b}	21.49^{c}	21.68^{c}	42.65^{d}	43.04^{d}	$2.15^{a,b}$	$2.02^{a,b}$
SGS	61.72^{c}	57.42^{c}	19.02^{d}	20.43^{d}	20.46^{c}	20.10^{c}	$35.93^{b,c,d}$	$38.95^{c,d}$	2.55^{b}	2.49^{b}
EGS	124.33^{d}	119.74^{d}	10.04^{c}	10.18^{c}	13.68^{b}	13.63^{b}	26.73^{b}	$28.36^{b,c}$	$1.40^{a,b}$	$1.28^{a,b}$

Table 3.12 Comparison of the Amounts of Copper Extracted from Grass and Sawdust Blend Samples Taken at t= 318 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.13^{a}	0.01^{a}	0.00^{a}	0.01^{a}	0.04^{a}	0.04^{a}	1.30^{a}	1.20^{a}	0.16^{a}	0.14^{a}
AGS	$52.3^{b,c}$	53.87^{c}	21.85^{d}	22.10^{d}	21.55^{c}	19.45^{c}	47.62^{d}	50.35^{d}	2.78^{d}	2.64^{d}
SGS	50.57^{b}	$52.95^{b,c}$	12.44^{c}	12.69^{c}	14.58^{b}	14.19^{b}	$32.51^{b,c}$	33.33^{c}	2.12^{c}	1.99^{c}
EGS	74.15	80.95	10.20^{b}	10.53^{b}	11.78^{b}	11.27^{b}	29.41^{b}	29.57^{b}	1.71^{b}	1.58^{b}

Table 3.13 Comparison of the Amounts of Copper Extracted from 0 Copper and Copper Acetate Amended Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blend Samples Taken at t= 0 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.13^{a}	0.11^{a}	0.03^{a}	0.02^{a}
AGS	66.72^{c}	64.59^{c}	15.14^{b}	15.47^{b}	15.61^{b}	16.31^{b}	$13.51^{c,d}$	$13.14^{b,c,d}$	0.95	0.84
UPS	2.99^{a}	2.39^{a}	0.60^{a}	0.66^{a}	0.31^{a}	0.289^{a}	15.50^{d}	14.97^{d}	0.61^{c}	0.54^{c}
APS	42.69^{b}	41.02^{b}	15.53^{b}	16.38	14.36^{b}	14.27^{b}	85.53	96.28	2.99^{d}	2.90^{d}
UCS	2.29^{a}	2.03^{a}	0.587^{a}	0.65^{a}	0.40^{a}	0.38^{a}	$6.47^{a,b,c}$	$6.46^{a,b}$	0.35^{b}	0.30^{b}
ACS	1.18^{a}	1.69^{a}	0.459^{a}	0.51^{a}	0.32^{a}	0.31^{a}	4.82^{a}	4.79^{a}	0.31^{b}	0.26^{b}

Table 3.14 Comparison of the Amounts of Copper Extracted from 0 Copper and Copper Acetate Amended Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blend Samples Taken at t= 105 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.07^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.03^{a}	0.02^{a}	0.67^{a}	0.64^{a}	0.08^{a}	0.07^{a}
AGS	27.85^{c}	27.00^{c}	5.29^{c}	5.98^{c}	21.49^{c}	21.68^{c}	42.65^{c}	43.04^{c}	2.15^{d}	2.02^{d}
UPS	1.04^{a}	0.83^{a}	0.32^{a}	0.36^{a}	0.00^{a}	0.00^{a}	15.93^{b}	15.73^{b}	0.98^{c}	0.89^{c}
APS	39.11^{d}	37.66^{d}	25.98^{d}	26.03^{d}	54.37	59.33	100.73	118.28	4.46^{f}	4.45^{f}
UCS	1.45^{a}	1.32^{a}	0.24^{a}	0.25^{a}	0.66^{a}	0.70^{a}	$9.85^{a,b}$	$9.60^{a,b}$	0.66^{b}	0.58^{b}
ACS	15.39^{b}	13.40^{b}	4.61^{b}	4.51^{b}	14.89^{b}	15.45^{b}	54.52^{d}	58.75^{d}	2.74^{e}	2.62^{e}

Table 3.15 Comparison of the Amounts of Copper Extracted from 0 Copper and Copper Acetate Amended Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blend Samples Taken at t= 318 Days Sampling by the Tessier Sequential Extraction Scheme.

Each fraction was measured twice for copper, first using Matrix-Matched Standards (MM) and then again using Matrix-Matched Standards Coupled with Standard Additions (MMSA).
Results are in mg l^{-1} of the Extractant.
Identical superscript lettering within each extracted fraction indicates non-significance at 95% confidence limits

Treatment	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA	MM	MMSA
UGS	0.13^{a}	0.01^{a}	0.00^{a}	0.01^{a}	0.04^{a}	0.04^{a}	1.30^{a}	1.20^{a}	0.16^{a}	0.14^{a}
AGS	52.53	53.87	21.85^{d}	22.10^{d}	$21.55^{b,c}$	19.45^{b}	47.62^{c}	50.35^{c}	2.78^{c}	$2.64^{b,c}$
UPS	2.52^{b}	2.20^{b}	0.57^{b}	0.61^{b}	1.41^{a}	1.51^{a}	22.04^{b}	21.48^{b}	$1.14^{a,b}$	$1.12^{a,b}$
APS	28.06	22.98	0.00^{a}	0.00^{a}	38.64^{d}	40.07^{d}	110.20^{e}	116.40^{e}	3.70^{c}	3.63^{c}
UCS	0.67^{a}	0.64^{a}	0.16^{a}	0.16^{a}	0.31^{a}	0.30^{a}	8.08^{a}	8.20^{a}	0.55^{a}	0.50^{a}
ACS	14.24	11.92	7.02^{c}	6.73^{c}	24.34^{c}	24.28^{c}	62.88^{d}	72.86^{d}	3.10^{c}	3.05^{c}

multiple comparison analysis showed that there were no significant differences between the two methods for any one treatment.

Occasionally a significant difference was found between the two methods for a particular fraction of a particular sample. One such example was the magnesium chloride extraction (Fraction 1) of grass/sawdust samples taken at t=318 days. Here, all effects and interactions were very highly significant ($p \leq 0.001$) and a significant difference was found between the two methods for the EGS, with the amount of copper determined increasing from 74.15 mgl^{-1} for the matrix-matched standards to 80.95 mgl^{-1} for the matrix-matched standards plus standard additions method. Interferences within the flame were therefore suppressing the signal to a significant extent in the first instance. Despite this, the two values obtained by both methods were still significantly different from those of all the other treatments, meaning that any conclusions drawn would remain the same. Similar patterns of behaviour were seen with other samples, including, but not exclusive to, the oxidisable fraction (Fraction 4) of the APS and also the reducible and oxidisable fractions of the same sample types taken at t=105 days.

Only on three occasions did significant differences mean that the conclusions drawn would have been altered. These were all for samples taken at t=318 days and occurred where there were actually no overall significant differences between the two methods for a particular sample. However, different patterns of significance between the other samples were still observed, depending on the method chosen. This is most clearly illustrated by the example of the

reducible fraction (Fraction 3). Here, where only matrix-matched standards were used, the AGS was not found to be significantly different from the ACS. However, the two treatments were found to be significantly different from each other where the standard additions technique was used; and this was in spite of the fact that no significant differences between the two methods for any one sample were demonstrated. Similar occurrences were also seen in the oxidisable fraction (Fraction 4) between the SGS and the EGS; and in the residual fraction (Fraction 5) of the AGS and the ACS.

To summarise, whilst flame interferences have been demonstrated in a few of the extractants, in the vast majority of cases no significant differences between the presence/absence of the use of standard additions were seen. Even on the three occasions where differences between samples depended on the method used, this hardly made any difference to overall trends within the data set as a whole. It was therefore concluded that to proceed with the statistical analysis of the original set of data would be perfectly appropriate and that, even if flame interferences had occurred, they would have taken place with such infrequency as to make little or no difference to the conclusions drawn. Simply put, the time, effort and expense of reanalysing every single sample using the standard additions method was not warranted.

3.2.3.2 Results of the Sequential Extraction Analysis of all Samples Using the Method of Tessier *et. al.* (1979)

Data for all extractions are presented in both mg kg^{-1} and as a percentage of the total amount of copper extracted from each sample (the percentage share). Data was not corrected for any losses in volatile solids. Data for the four treatments of the grass/sawdust blend samples were analysed separately as one group (Group A) and the zero copper (unamended) and copper acetate treatments of the grass/sawdust; pig slurry/sawdust and sewage sludge cake/sawdust samples were analysed as another (Group B).

Within both groups, data were analysed fraction by fraction by Analysis of Variance (ANOVA) using Tukey's method of multiple comparisons between means at the 95% confidence interval. The software program Genstat® (8^{th} and 9^{th} Editions) was used for all statistical analyses and the variates analysed were:

1. Amount of extractable Cu (mg kg^{-1}) for that fraction at t=0 days.
2. Percentage of the overall total extractable amount at t=0 days
3. Change between t=105 days and t=318 days in the amount of extractable Cu for that fraction (mg kg^{-1}).
4. Change between t=105 days and t=318 days in extractable Cu for that fraction expressed as a percentage of the overall total extractable amount.
5. Amount of extractable Cu (mg kg^{-1}) for that fraction at t=105 days.
6. Percentage of the overall total extractable amount at t=105 days

7. Amount of extractable Cu (mg kg^{-1}) for that fraction at t=318 days.

8. Percentage of the overall total extractable amount at t=318 days.

Results for t=0 days cannot be compared directly with samples taken t=105 and 318 days using Analysis of Variance (ANOVA) as the data were obtained from 3 separate samples of each of the initial starting materials only and not from the individual boxes which were prepared for incubation. Data for t=0 days were not therefore obtained from the same randomised and blocked samples which are the requirements of ANOVA tests (Townend, 2005). Therefore it was not possible to measure the change between t=0 days and t= 105 days in the amount of extractable Cu for the various fractions in the way that it was possible to measure the change between t=105 days and t=318 days.

Group A: Grass/Sawdust Blend - Unamended, Copper Acetate, Copper Sulphate and Copper EDTA Treatments

Data for these extractions are given in Figures 3.3 to 3.4 and Tables 3.16 – 3.19.

a) Fraction 1 - Magnesium Chloride Extract

For the amounts of copper extracted, all treatment effects were found to be very highly significant at the start of the experiment (t=0 days). No copper was detected in the UGS, significantly more was extracted from

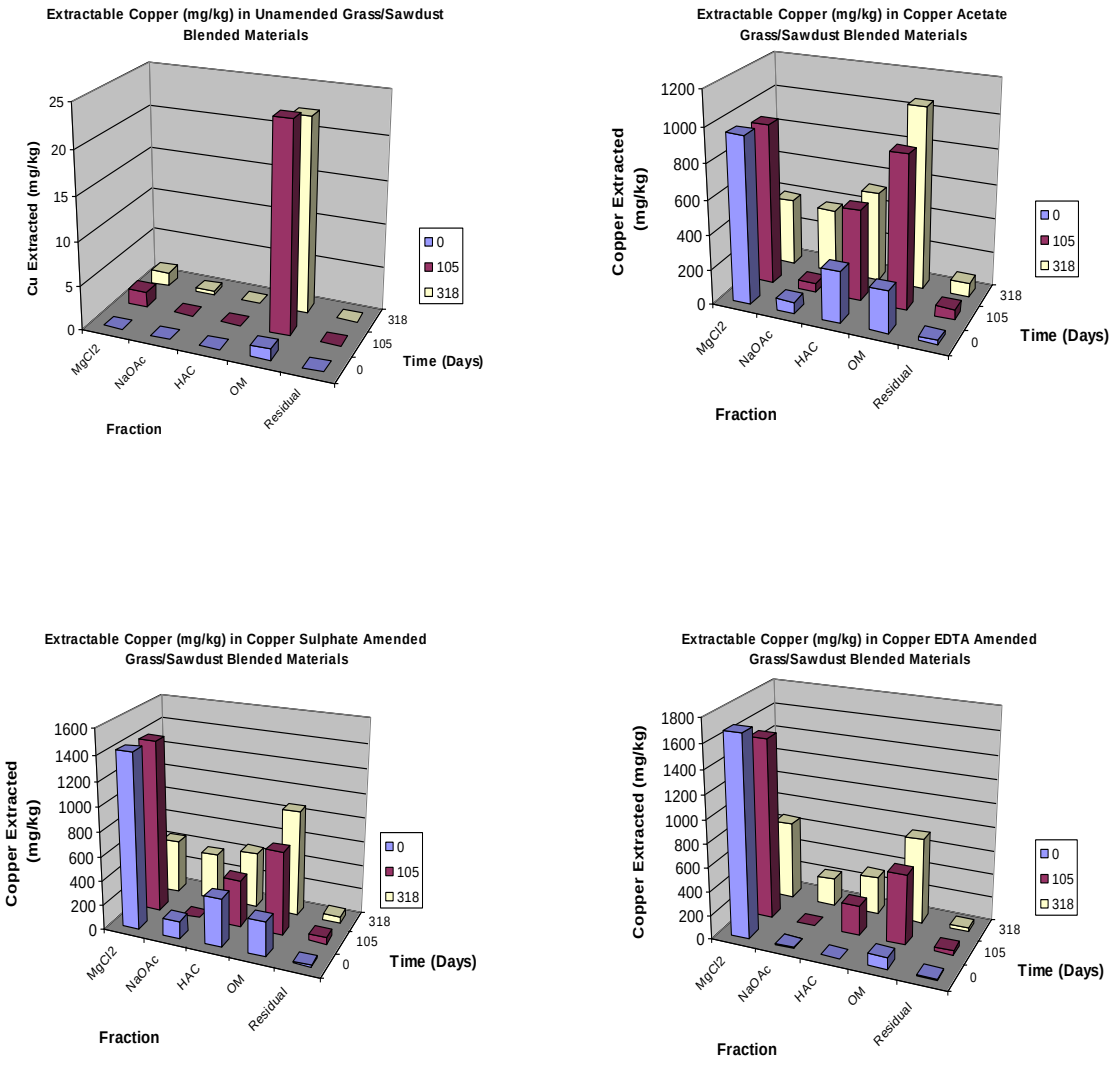


Fig 3.3 Changes Over Time in the Distribution of Extractable Copper ($mgkg^{-1}$) Within Copper Treated Grass/Sawdust Blended Materials Using Tessier *et. al.* (1979) Sequential Extraction Scheme.

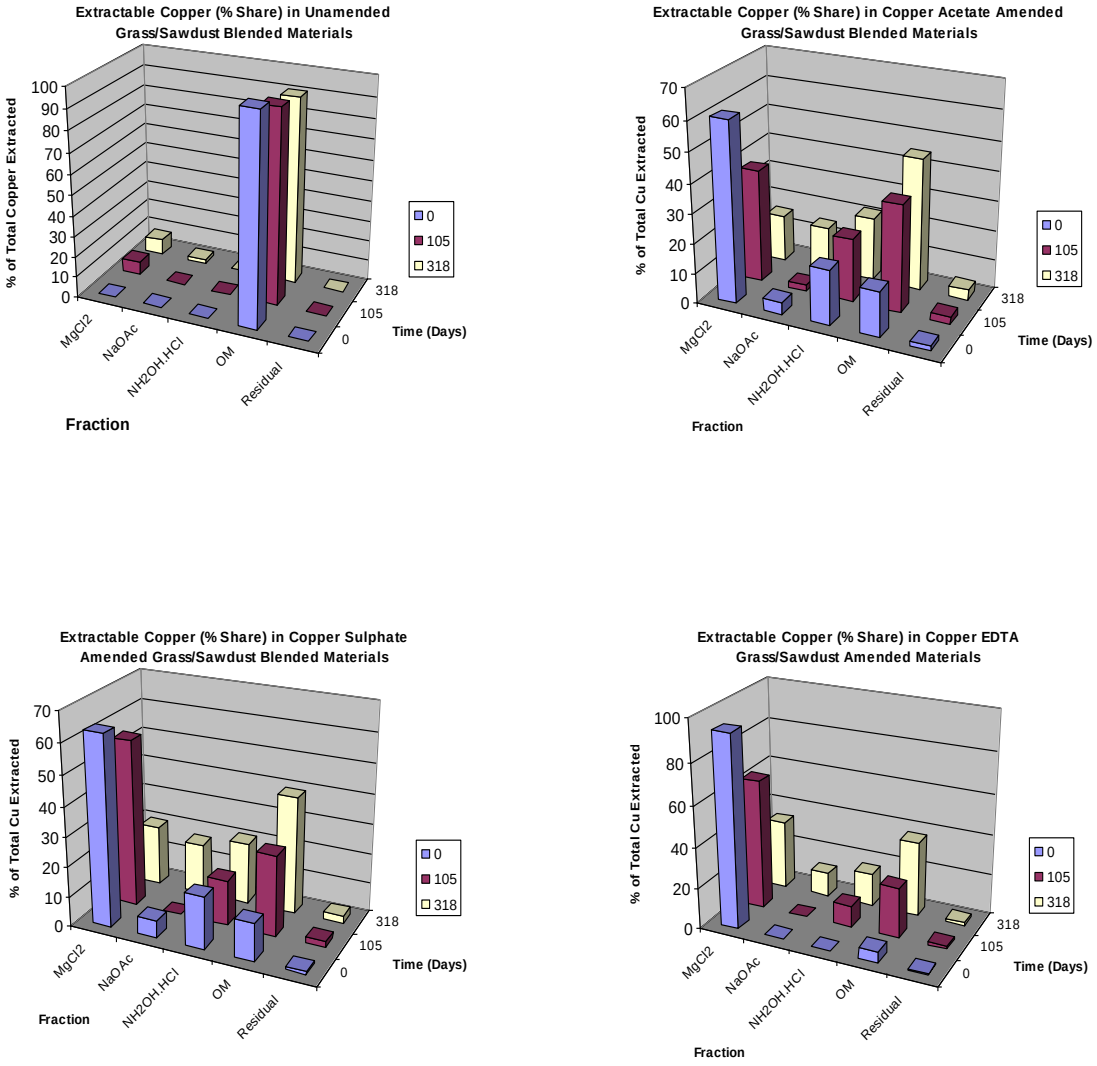


Fig 3.4 Changes Over Time in the Distribution of Extractable Copper (% Share of the Total Amount Extracted) Within Copper Treated Grass/Sawdust Blended Materials Using Tessier *et. al.* (1979) Sequential Extraction Scheme.

Table 3.16 Copper Extracted from the Grass and Sawdust Blends (t=0 Days Samples) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	0.00	0.00	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	1.30	100	0.00^{a}	0.00^{a}	1.30	N/A
AGS	954^{a}	60.40^{a}	$66.89^{a,b}$	4.09^{b}	294.7^{b}	18.58^{b}	240.0	15.33	24.39^{c}	1.60^{b}	1580^{a}	N/A
SGS	1423^{a}	63.25^{a}	134.52^{b}	5.93^{b}	392.3^{b}	17.41^{b}	283.7	12.62	$17.82^{b,c}$	$0.80^{a,b}$	1795^{a}	N/A
EGS	1679^{a}	93.65	7.72^{a}	0.43^{a}	0.00^{a}	0.00^{a}	95.5	5.32	$10.72^{a,b}$	0.60^{a}	2251^{a}	N/A

* Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated.

Table 3.17 Copper Extracted from the Grass and Sawdust Blends (t=105 Days Samples) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signify no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	1.68	7.60	0.00^{a}	0.00^{a}	0.00	0.00	23.7	92.40	0.00	0.00	25.34	N/A
AGS	929.36^{a}	37.33	48.39^{a}	1.75^{a}	524.72^{b}	21.65	877.7^{a}	36.74^{a}	60.48^{a}	2.52^{b}	2440.66^{a}	N/A
SGS	$1402.38^{a,b}$	55.77^{a}	0.00^{a}	0.00^{a}	$382.18^{a,b}$	15.11	687.0^{a}	26.97^{a}	53.48^{a}	$2.14^{a,b}$	2516.08^{a}	N/A
EGS	1517.32^{b}	63.40^{a}	0.00^{a}	0.00^{a}	254.09^{a}	10.63	587.4	24.53^{a}	34.37	1.44^{a}	2393.13^{a}	N/A

* Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated.

Table 3.18 Copper Extracted from the Grass and Sawdust Blends (t = 318 Days Samples) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signify no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	1.63	7.65^{a}	0.37^{a}	1.88^{a}	0.05	0.13	22.37	90.34	0.00	0.00	24.42	N/A
AGS	390.48^{a}	$15.85^{a,b}$	372.07^{a}	14.67^{a}	518.50^{b}	21.47^{a}	1050.97^{b}	44.49^{a}	83.13	3.51^{b}	2415^{a}	N/A
SGS	$441.69^{a,b}$	20.11^{b}	384.09^{a}	17.06^{a}	$459.28^{a,b}$	20.78^{a}	$865.01^{a,b}$	39.53^{a}	55.30^{a}	$2.52^{a,b}$	2205^{a}	N/A
EGS	660.54^{b}	33.61	233.52^{a}	11.79^{a}	318.45^{a}	16.16	721.93^{a}	36.80^{a}	32.34^{a}	1.64^{a}	1967^{a}	N/A

* Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated.

Table 3.19 Comparison of the Changes in Extractable Copper from the Grass and Sawdust Blends Between t = 105 and t = 318 Days as Extracted by the Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment *	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracte d $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	0.05	-0.05	-0.37^{b}	-1.88^{b}	-0.05^{a}	-0.13^{b}	1.29^{a}	2.06^{b}	0.00^{b}	0.00^{b}	0.92^{a}	N/A
AGS	538.89^{a}	21.48	$-323.68^{a,b}$	$-12.92^{a,b}$	6.22^{a}	0.172^{b}	-173.27^{a}	$-7.75^{a,b}$	-22.64^{a}	-0.99^{a}	25.52^{a}	N/A
SGS	960.70^{b}	35.66	-384.09^{a}	-17.06^{a}	-77.10^{a}	-5.66^{a}	-186.97^{a}	-12.56^{a}	$-1.82^{a,b}$	$-0.37^{a,b}$	310.72^{a}	N/A
EGS	$856.79^{a,b}$	29.79	$-233.5^{a,b}$	$-11.79^{a,b}$	-64.36^{a}	-5.54^{a}	-134.57^{a}	-12.26^{a}	2.03^{b}	-0.20^{b}	426.37^{a}	N/A

- Key: UGS = untreated; AGS = copper acetate treated; SGS = copper sulphate treated; EGS = copper-EDTA treated

the AGS (954 mgkg^{-1}) and more still in the SGS and EGS treatments (1423 and 1679 mg kg^{-1} respectively), although there was no significant difference between these last two treatments. In terms of percentage share, the EGS contained the most – 93.65% of the total amount extractable from the material. This was significantly different from all the others, showing that chelated copper was not readily converted to other forms.

By t=105 days a small amount (1.68 mg kg^{-1}) of magnesium chloride extractable copper was found in the control material, produced as a result of the breakdown of organically bound forms within the oxidisable fraction of the materials. However, this was still very small compared with the other treatments all of which had decreased slightly in their magnesium chloride extractable copper content, to the extent that the EGS was now no longer significantly different from the SGS. This was also true in terms of the percentage share data. The control had an increase in extractable copper – from 0.00% to 7.6% of the total extractable amount - and in the three treatments, the proportion of copper extractable by magnesium chloride had all decreased. Treatment effects were very highly significant ($p \leq 0.001$) for both the amounts and proportions of copper extracted.

At the end of the composting process (t=318 days) treatment effects were still very highly significant ($p \leq 0.001$). Copper in the control material remained essentially constant at 1.6 mg kg^{-1} (7.65%) whilst the copper treatment all continued to decrease in both the amount and proportion of extractable copper. The extent to which the different treatments changed during this

second phase of the process depended on the copper treatment. In terms of the amounts of copper extracted, the only significant difference observed was that between the SGS and AGS. Greater variation though was seen with the percentage share data. Here, the SGS changed the most, decreasing by 35.66 percentage points. In all the copper amended treatments, conversion to sodium acetate extractable and organically bound forms seems the most likely explanation (see Fig. 3.3).

b) Fraction 2 – Sodium Acetate Extract

Treatment effects were highly significant for both the amount of extractable copper ($p=0.003$) and the proportion of extractable copper ($p=0.002$). The control material contained no sodium acetate extractable copper and the EGS was only found to contain a small amount (7.72 mg kg^{-1} / 0.43%), presumably because most of the copper EDTA was still in the magnesium chloride extractable form. The AGS and SGS treatments contained significantly more (66.89 and 134.52 mg kg^{-1} respectively) though these were not significantly different from each other.

By t=105 days, treatment effects were no longer significant for either the amounts ($p=0.240$) or the proportions of sodium acetate extractable copper ($p=0.216$). This was because, in most instances, copper was not measurable in this fraction. Only the AGS treatment contained detectable amounts of copper (48.39 mg kg^{-1}). Overall, it appeared that sodium acetate extractable

copper had decreased during the first phase of the experiment, possibly owing to its conversion to other forms.

During the second phase of the experiment all treatments had increased their amounts of sodium acetate extractable copper and treatment effects were still not significant (p=0.054), but only just. The UGS had increased to only 0.37 mg kg^{-1} (1.88%), but the copper amended treatments had all increased by more than 200 mg kg^{-1} (ranging from 11.79 – 17.06%), although no treatment was found to be significantly different from another. These increases reflected large decreases in magnesium chloride extractable copper over this final phase. Overall, the sodium acetate extractable fraction plays a much smaller part in the sequential extraction scheme compared with the magnesium chloride extractable fraction.

c) Fraction 3 – Hydroxylamine Hydrochloride Extract ('Reducible' Fraction)

No copper held within the reducible fraction was found in either the UGS or the EGS as copper in the control was all bound within the organic fraction and copper in the EGS was mainly in magnesium chloride extractable forms. The SGS and AGS treatments contained significantly more (294.7 and 392.3 mg kg^{-1} respectively) but were not significantly different from each other. These relationships between the treatments were also mirrored in the percentage share data and, for both cases, treatment effects were very highly significant.

By t=105 days treatment effects were still very highly significant. No change had occurred in the control material and only a very small decrease was seen in the copper sulphate treatment. However, noticeable changes had taken place within the other treatments, both of which had seen large increases in the amounts and proportions of copper within the reducible fraction (although it is not possible to ascertain whether these changes are significant or not).

Treatment effects at the end of the composting period (t=318 days) were still very highly significant for both the amounts and proportions of copper extractable. A very small increase of 0.05 mg kg^{-1} (0.13%) was seen in the control material and a small decrease to 518.5 mg kg^{-1} was seen in the AGS but as this amounted to a decrease of only 0.172 percentage points this was probably not a significant change. The SGS and EGS increased to 459.3 and 318.4 mg kg^{-1} respectively although these were not significantly different from each other. Analysis of the extent to which the amounts of extractable copper in these treatments changed over the second phase of the experiment failed to detect any significant changes (treatment effects were not significant as p=0.301). However, real differences were seen in the changes of proportions of extractable copper. The control (increased by 0.13 percentage points) and the AGS (decreased by 0.17 percentage points) were not significantly different from each other, but they were different from the SGS and EGS which correspondingly increased significantly by 5.66 and 5.54 percentage points correspondingly.

d) Fraction 4 – ‘Oxidisable Fraction’

Treatment effects at t=0 days were very highly significant ($p \leq 0.001$) for both the amounts of extractable copper and the percentage share data. The amounts of extractable copper in the various treatments were of the order: UGS < EGS < AGS < SGS. Even within these uncomposted materials it is clear from the results that some of the additional copper was very quickly converted to these organically bound forms, particularly when added as copper acetate or copper sulphate. Even with the EGS, though it was predominantly found within the magnesium chloride fraction, a small amount (95.5 mg kg^{-1} / 5.32%) still became organically bound demonstrating that, even in uncomposted materials, organic sites which effectively compete with the EDTA to chelate the copper were present right at the start.

Considered in terms of the proportion of extractable copper which was present in the oxidisable fraction, all treatments were significantly different from one another and were of the order: EGS < SGS < AGS < UGS. Copper in the control was found entirely in the oxidisable fraction (100%), whereas in the EGS it amounted to only 5.32% of the total extractable amount.

Treatment effects at t=105 days were still very highly significant. All treatments and the control had increased in the amounts of copper held within their oxidisable fractions. Increases for all the treatments are too large to be credited entirely to the conversion of copper from other forms (e.g. movement from the magnesium chloride extractable fraction). They must also be due, in part, to the composting process itself which results in a decrease in the volume of the materials. This is supported by the fact that copper within the

oxidisable fraction, as a proportion of the total amount extractable within the control, had actually decreased from 100% to 92.4% and yet the amount extractable had increased from 1.3 mg kg^{-1} to 23.7 mg kg^{-1}. Increases in the percent share of copper in this fraction for the copper amended treatments were substantial and would be attributed to the conversion of copper from other forms (principally magnesium chloride extractable ones).

By the end of the experiment at t=318 days, treatment effects were still very highly significant ($p \leq 0.001$). The control was largely unchanged from the amount it held at t=105 days, while the copper amended treatments increased in the amounts of copper extracted from the oxidisable fractions to 1051, 865 and 722 mg kg^{-1} for the AGS, SGS and EGS treatments respectively. In terms of the proportions they contained, none of the copper amended treatments differed significantly from another. The control still contained the largest proportion of copper within the oxidisable fraction (90.34%).

e) Fraction 5 – Residual Fraction

It is clear from the data that at the start of the experiment residual forms play an almost insignificant part in the distribution of copper within the materials and, in most cases, is the smallest fraction of all. Treatment effects for the amounts of extractable copper were highly significant ($p=0.002$) and, whilst no copper was detectable in this fraction in the control material, some 10.7 to 24.4 mg kg^{-1} were detected in the copper amended treatments, though few significant differences were seen between them. As these copper amended

treatments were significantly different from the control, the copper in this fraction must have come from the treatment itself, once more demonstrating that the additional copper is very quickly converted to these other chemical forms.

Fewer significant differences were found between the treatments when the copper data was considered in terms of the proportions of the total extractable amount. Only the AGS was significantly different from the others with 1.6% of its copper being held in residual forms compared with 0.0% for the control and 0.6% for the EGS.

By t=105 days treatment effects were very highly significant for both the amounts which could be extracted and as proportions of the total amount extractable from the materials ($p \leq 0.001$). Residual forms of copper had increased considerably in all treatments except the control material although they still remained a very small percentage of the overall extractable amounts.

Some small changes had taken place during the second phase of the experiment. By t=318 days the EGS appeared to have decreased its residual copper content by 2.03 mg kg^{-1} (0.20%); the AGS and SGS increased by 22.64 and 1.82 mg kg^{-1} (0.99% and 0.37%) and the control remained unchanged. It is not possible though to determine whether or not the residual copper contents at t= 318 days are significantly different from those at t=105 days. Certainly, differences in the extent to which the different treatments

changed were very small and only the AGS demonstrated significantly different behaviour.

f) Total Copper Extracted (Sum of Fractions 1-5)

Results for the total amount of copper extracted can be found in Fig.3.5.

At t=0 days, the UGS contained a very small amount of copper (only 1.3 mg kg^{-1}), while in the copper amended materials much more was extracted. Copper had been applied to the materials at a rate of 1000 mg Cu kg^{-1} materials on a fresh weight basis. Taking the moisture content into account meant that it had been applied at a rate of 1871 mg kg^{-1}. Recoveries ranged from a low of 1580 mg kg^{-1} for the AGS to a high of 2251 mg kg^{-1} for the EGS. However, variation within the data meant that these were not significantly different from the calculated application rate.

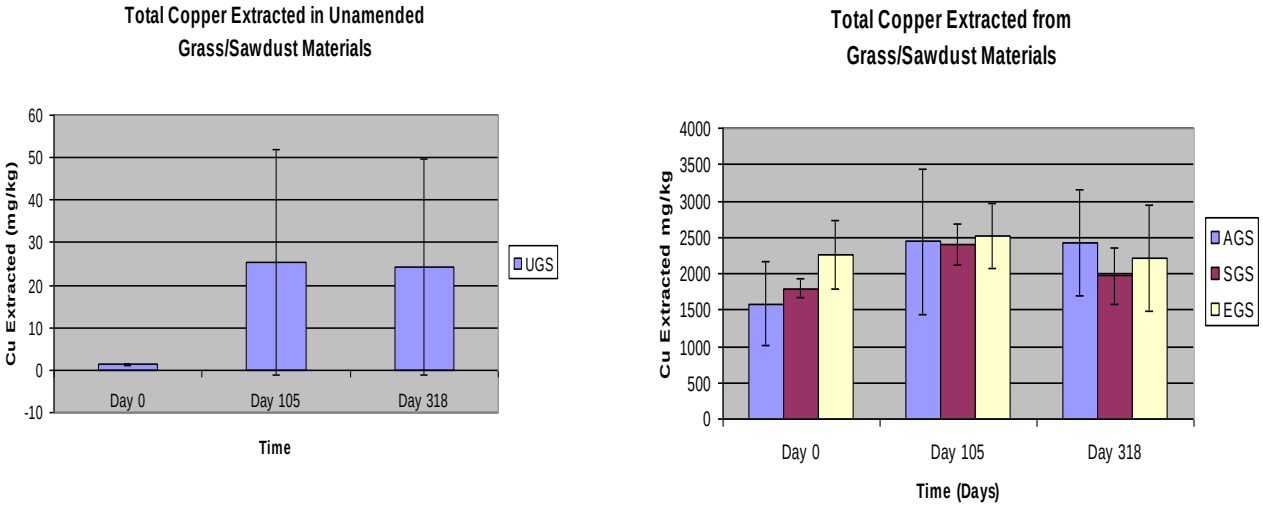


Fig. 3.5 Total Copper (Sum of 5 Fractions) Extracted from Grass/Sawdust Materials by the Tessier et al (1979) Sequential Extraction Scheme. Error bars are ± 95% Confidence Intervals

The trend during the composting process appeared to be one of an increase in the total amount of copper extracted from the samples. However, it was only with the SGS materials that any increase (during the first phase) appeared to be significant. The UGS, during the first phase of the experiment, also appeared to show a large increase in extractable copper, but large variation within the data meant that this did not prove to be significant at the 95% confidence interval. No significant changes were seen during the second phase either and total extractable copper in all treatments remained broadly unchanged.

This lack of change in total extractable copper also reflects the lack of change in organic matter content (as determined by loss on ignition), details of which are given in Section 3.6. No measurable changes in the organic matter contents of the samples were found.

Group B: Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends – Unamended and Copper Acetate Amended Treatments.

Data for these extractions are given in Figures 3.6 to 3.7 and Tables 3.20 – 3.23.

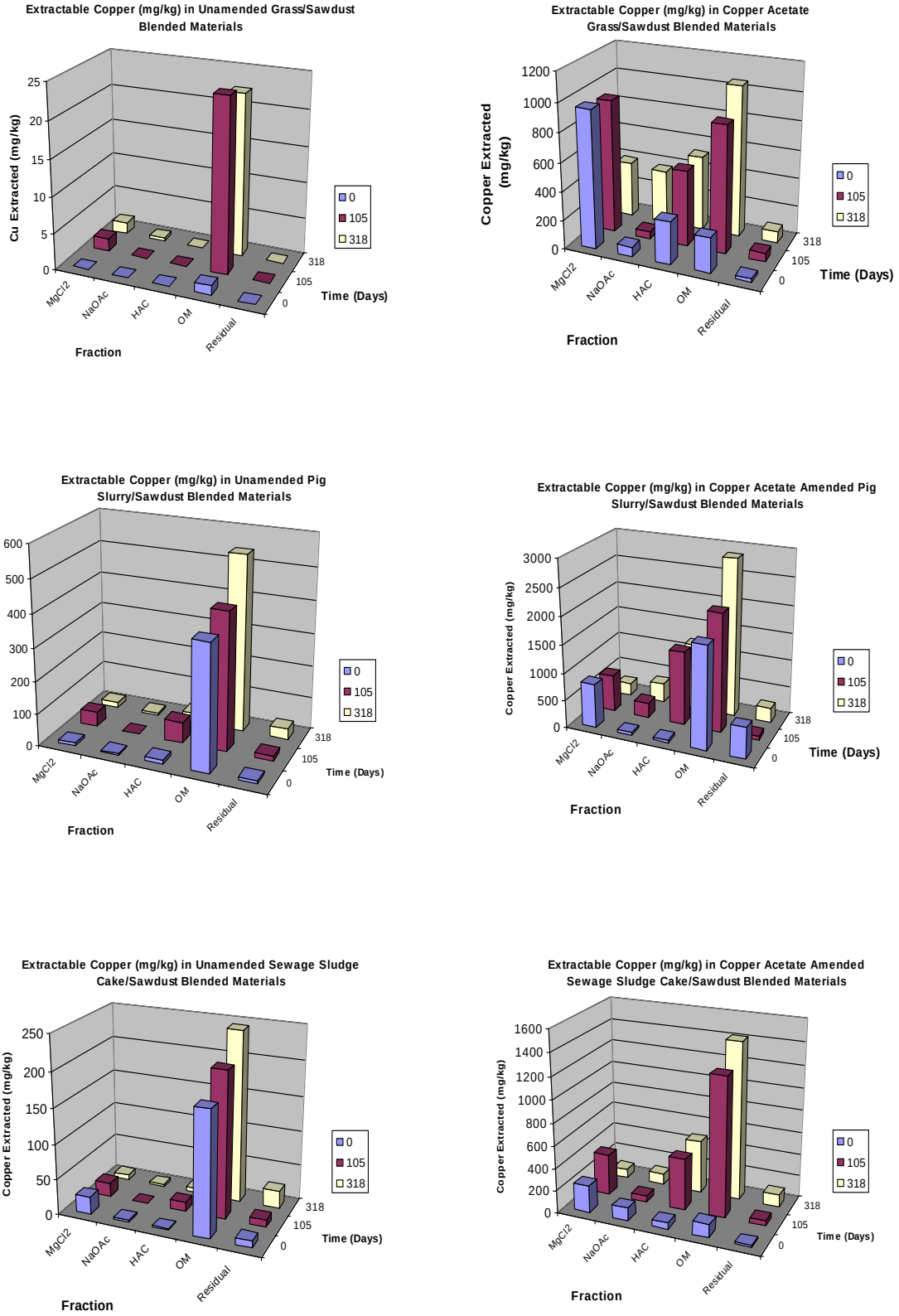
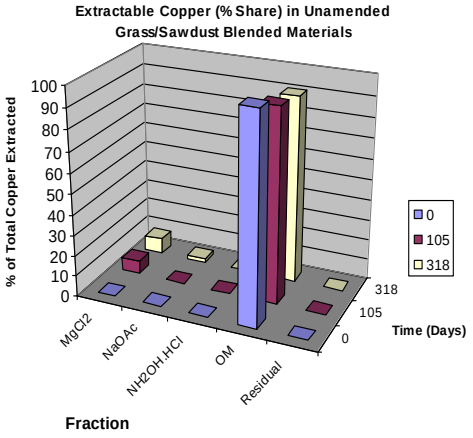
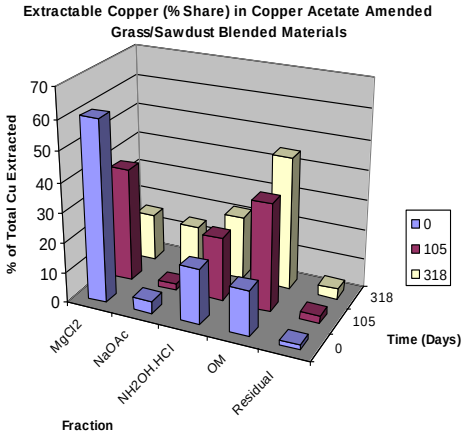
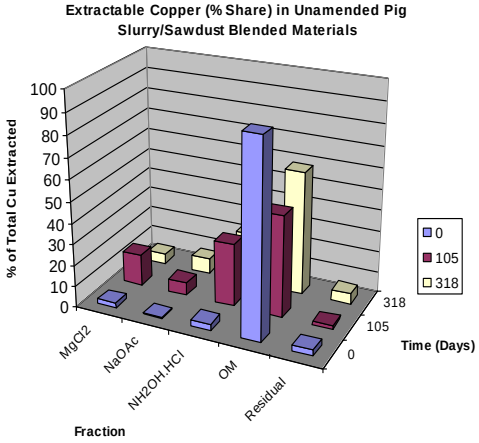
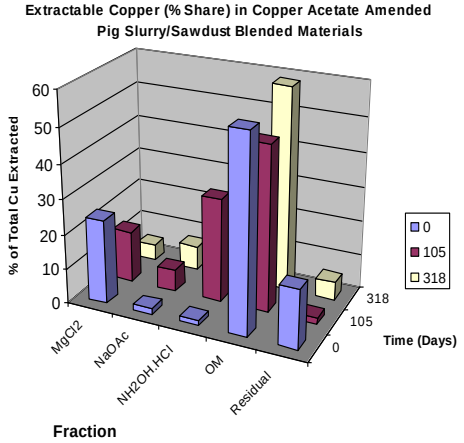


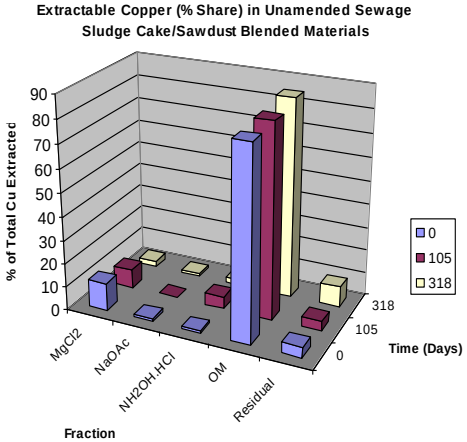
Fig. 3.6 Changes Over Time in the Distribution of Extractable Copper ($mgkg^{-1}$) Within Unamended and Copper Acetate Treated Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Materials Using Tessier *et. al.* (1979) Sequential Extraction Scheme.











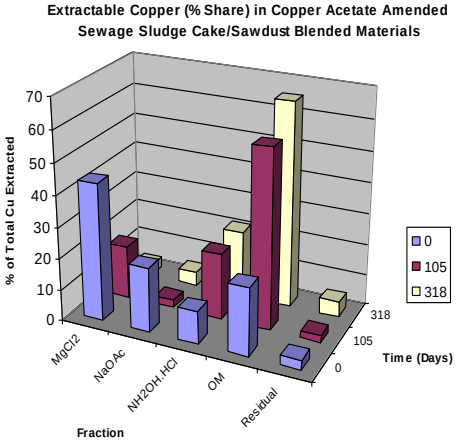


Fig 3.7 Changes Over Time in the Distribution of Extractable Copper (% Share of the Total Amount Extracted) Within Unamended and Copper Acetate Treated Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Materials Using Tessier *et. al.* (1979) Sequential Extraction Scheme.

Table 3.20 Copper Extracted from the Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends (t=0 Days) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	1.3	100.00	0.00^{a}	0.00^{a}	1.3^{a}	N/A
AGS	954.0^{b}	60.40	66.89^{b}	4.09^{b}	294.73	18.58	240.0^{b}	15.33	24.4^{a}	$1.60^{a,b}$	1580	N/A
UPS	9.14^{a}	2.19^{a}	3.30^{a}	0.79^{a}	13.23^{a}	3.17	381.8	91.31	10.6^{a}	$2.55^{b,c}$	$418^{b,c}$	N/A
APS	790.2^{b}	24.09	51.00^{b}	$1.55^{a,b}$	49.93^{a}	1.52^{b}	1831.0	55.91	555.0	16.92	3277	N/A
UCS	25.06^{a}	11.66	2.91^{a}	$1.34^{a,b}$	2.24^{a}	$1.06^{a,b}$	$175.7^{a,b}$	81.44	9.6^{a}	4.50^{c}	$215^{a,b}$	N/A
ACS	244.2	43.99	113.64	20.66	58.26^{a}	10.59	119.7^{a}	21.78	16.5^{a}	$2.99^{b,c}$	552	N/A

Key: UGS = Unamended grass/sawdust blend; AGS = Copper Acetate Treated grass/sawdust blend; UPS = Unamended Pig Slurry/Sawdust Blend; APS = Copper Acetate Treated Pig Slurry/Sawdust Blend; UCS = Untreated Sewage Sludge Cake/Sawdust Blend and ACS = Copper Acetate Treated Sewage Sludge Sawdust Blend.

Table 3.21 Copper Extracted from the Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends (t=105 Days) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	1.7^{a}	7.60^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	23.7^{a}	92.40^{c}	0.00^{a}	0.00	25^{a}	N/A
AGS	929.4^{c}	37.33	48.39^{a}	1.76^{a}	524.7^{b}	21.65^{a}	877.7	36.74	$60.48^{c,d}$	$2.52^{a,b}$	2441^{b}	N/A
UPS	43.7^{a}	8.03^{a}	0.00^{a}	0.00^{a}	63.9^{a}	11.76	419.9^{b}	77.38^{b}	15.45^{b}	2.84^{a}	543^{a}	N/A
APS	$662.8^{b,c}$	14.80^{a}	275.61	6.10	1333.4	29.81	2127.3	47.64^{a}	73.96^{d}	1.66^{b}	4473	N/A
UCS	19.9^{a}	7.68^{a}	0.61^{a}	0.27^{a}	13.0^{a}	5.13	$207.3^{a,b}$	$82.74^{b,c}$	$10.53^{a,b}$	4.19	251^{a}	N/A
ACS	$366.6^{a,b}$	16.83^{a}	53.50^{a}	2.40^{a}	457.7^{a}	21.08^{a}	1239.9	57.46^{a}	48.04^{c}	$2.23^{a,b}$	2166^{b}	N/A

Key: UGS = Unamended grass/sawdust blend; AGS = Copper Acetate Treated grass/sawdust blend; UPS = Unamended Pig Slurry/Sawdust Blend; APS = Copper Acetate Treated Pig Slurry/Sawdust Blend; UCS = Untreated Sewage Sludge Cake/Sawdust Blend and ACS = Copper Acetate Treated Sewage Sludge Sawdust Blend.

Table 3.22 Copper Extracted from the Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends (t=318 Days) by Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	1.6^{a}	7.65^{a}	0.4^{a}	1.88^{a}	0.00^{a}	0.13^{a}	22^{a}	90.34	0.00^{a}	0.00	24^{a}	N/A
AGS	390.5^{c}	15.85	372.1^{b}	14.67^{b}	518.5^{b}	21.47^{c}	1051	44.49^{c}	83.13^{b}	3.51^{a}	2415^{b}	N/A
UPS	15.9^{a}	2.54^{a}	6.9^{a}	1.10^{a}	29.6^{a}	4.71^{b}	541^{b}	86.23^{c}	33.98^{a}	5.42^{b}	627^{a}	N/A
APS	$229.2^{b,c}$	4.67^{a}	350.5^{b}	$7.10^{a,b}$	1199.6	24.43^{c}	2853	$58.52^{a,b}$	256.60	5.27^{b}	4888	N/A
UCS	7.6^{a}	2.59^{a}	3.2^{a}	1.11^{a}	6.8^{a}	$2.34^{a,b}$	$244^{a,b}$	85.03^{c}	25.63^{a}	8.94	287^{a}	N/A
ACS	$76.9^{a,b}$	3.50^{a}	$97.8^{a,b}$	$4.40^{a,b}$	471.9^{b}	21.48^{c}	1417	65.55^{b}	110.16^{b}	$5.07^{a,b}$	2174^{b}	N/A

Key: UGS = Unamended grass/sawdust blend; AGS = Copper Acetate Treated grass/sawdust blend; UPS = Unamended Pig Slurry/Sawdust Blend; APS = Copper Acetate Treated Pig Slurry/Sawdust Blend; UCS = Untreated Sewage Sludge Cake/Sawdust Blend and ACS = Copper Acetate Treated Sewage Sludge Sawdust Blend.

Table 3.23 Comparison of the Changes in Extractable Copper from the Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends Between t = 105 and t = 318 Days as Extracted by the Tessier *et. al.* (1979) Sequential Extraction Scheme. Results Given as mg Cu per kg of material and as percentages of the sum total of copper extracted. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Treatment*	Fraction 1 Magnesium Chloride Extractable		Fraction 2 Sodium Acetate Extractable		Fraction 3 Hydroxylamine Hydrochloride Extractable		Fraction 4 'Oxidisable'		Fraction 5 'Residual'		Total Extracted	
	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share	Cu Extracted $mgkg^{-1}$	Cu Extracted % Share
UGS	0.00^{a}	-0.05^{a}	-0.37^{b}	$-1.88^{a,b}$	-0.05^{a}	-0.13^{a}	1.3^{a}	2.06^{c}	0.00^{b}	0.00^{c}	0.92^{a}	N/A
AGS	538.9^{c}	21.48	-323.68^{a}	-12.92^{a}	6.22^{a}	$0.17^{a,b}$	-173.3^{a}	$-7.75^{a,b}$	$-22.64^{a,b}$	-0.99^{c}	25.52^{a}	N/A
UPS	27.8^{a}	5.48^{b}	-6.90^{b}	$-1.10^{a,b}$	34.33^{a}	7.05^{d}	-121.0^{a}	$-8.85^{a,b}$	-18.53^{b}	-2.58^{b}	-84.34^{a}	N/A
APS	$433.6^{b,c}$	$10.13^{b,c}$	$-74.85^{a,b}$	$-1.00^{a,b}$	133.76	$5.38^{c,d}$	-725.2	-10.89^{a}	-182.64	$-3.62^{a,b}$	-415.37	N/A
UCS	12.3^{a}	$5.09^{a,b}$	-2.62^{b}	-0.84^{b}	6.18^{a}	$2.79^{b,c}$	-36.4^{a}	$-2.29^{b,c}$	-15.10^{b}	-4.75^{a}	-35.60^{a}	N/A
ACS	289.6^{b}	13.33^{c}	$-44.27^{a,b}$	$-2.00^{a,b}$	-14.23^{a}	-0.40^{a}	-176.9^{a}	-8.09	-62.12^{a}	-2.84^{b}	-7.90^{a}	N/A

Key: UGS = Unamended grass/sawdust blend; AGS = Copper Acetate Treated grass/sawdust blend; UPS = Unamended Pig Slurry/Sawdust Blend; APS = Copper Acetate Treated Pig Slurry/Sawdust Blend; UCS = Untreated Sewage Sludge Cake/Sawdust Blend and ACS = Copper Acetate Treated Sewage Sludge Sawdust Blend.

a) Fraction 1 - Magnesium Chloride Extract

At the start of the experiment all effects and interactions were very highly significant ($p \leq 0.001$). Each of the unamended blends (controls) contained measurable amounts of copper - although these did not differ significantly from each other - and all contained significantly less copper than the copper acetate amended blends. Copper content within the amended materials varied somewhat. The AGS (954.0 mg kg^{-1}) and APS (790.2 mg kg^{-1}) did not differ significantly from each other, while the ACS contained significantly less (244.2 mg kg^{-1}), which suggested that much more of the added copper had quickly converted to other, less available forms. In terms of the percentage share of extractable copper found within this fraction, the relationships between the treatments changed slightly. Both the pig slurry/sawdust and the grass/sawdust controls contained very similar proportions of copper (2.19% and 0.0% accordingly) and did not differ significantly from each other. The UCS contained significantly more (11.66%). All however were significantly different from the copper acetate amended materials. These all differed significantly from each other and were in the order APS < ACS < AGS, the latter containing 60.40% of copper in magnesium chloride extractable forms.

By t=105 days only treatment effects were still very highly significant ($p \leq 0.001$), though blend and blend x treatment interactions were significant ($p=0.017$ and 0.012 respectively). None of the three control materials, nor the ACS were found to differ significantly in the amounts of magnesium chloride extractable copper

they contained, despite the fact that the difference between the UGS and the ACS was quite large (364.9 mg kg^{-1}). This lack of measurable significant difference may have been due to large variations within the data contributing to the rather high value of the CV (39.6%).

During this first phase of the experiment both the UGS and UPS appeared to increase both in the amounts and the percentage share of magnesium chloride extractable copper they contained whilst the AGS and APS appeared to decrease. The sewage sludge cake/sawdust treatments appeared to behave in an opposite manner, with the UCS decreasing in extractable copper and the ACS. However, whilst some of these changes appear to be substantial, it is not possible to determine to what extent they are statistically significant.

By the end of the experiment at t=318 days blend effects were highly significant (p=0.006), treatment effects very highly significant ($p \leq 0.001$) and blend x treatment interactions highly significant (p=0.004). Significant differences were seen between the amounts of extractable copper found in the UGS and UPS. However, no such differences were seen between the UCS and ACS materials. With the exception of the UGS which saw no changes between t=105 days and t=318 days, all of other materials appeared to decrease in the amounts of magnesium chloride extractable copper they contained. Corresponding increases in extractable copper were seen, primarily in the oxidisable fraction, secondarily in the residual fraction and thirdly, to a much smaller extent, the sodium acetate

extractable fraction. The extent to which the various materials changed during this second phase depended on the treatment. No significant differences were seen between the unamended materials whilst the APS and ACS changed significantly more than the unamended (though they were not different from each other) and the AGS changed the most (decrease of 538.9 mg kg^{-1}) but was not significantly different from the APS.

In terms of the proportions of copper held within this fraction, blend effects were found to be very highly significant ($p \leq 0.001$), treatment effects significant ($p=0.017$) but interactions were not significant ($p=0.099$). Only between the unamended (7.65%) and the AGS (15.85%) was there a significant difference. No other differences were seen between any of the treatments – amended or otherwise, indicating that, for at least the pig slurry/sawdust and sewage sludge cake/sawdust materials, the magnesium chloride extractable fraction had reached some kind of equilibrium with the other fractions. The extent to which the various materials changed during this second phase depended on the treatment. The AGS and ACS decreased more than the unamended materials with respect to the proportion of copper held within the fraction. However, no significant differences were seen between the two treatments of the pig slurry/sawdust blend.

b) Fraction 2 – Sodium Acetate Extract

At t=0 days, blend effects and blend x treatment interactions were significant ($p=0.018$ and 0.020 respectively) and treatment effects very highly significant ($p\leq0.001$) for the quantities of copper removed at this stage in the sequential extraction scheme. Reasonable consistency was seen amongst the blends when averaged across the two treatments (33.45 mg kg^{-1} for the grass/sawdust blend; 27.15 mg kg^{-1} for the pig slurry/sawdust blend and 58.27 mg kg^{-1} for the sewage sludge cake/sawdust blend) and only the difference between the pig slurry/sawdust and sewage sludge cake/sawdust materials was significant.

None of the control materials were found to contain significantly different amounts of sodium acetate extractable copper and all were significantly different from the copper acetate amended materials. Amongst the copper acetate treated materials, the ACS stood out as containing significantly more extractable copper (113.6 mg kg^{-1}; 20.66%) than all the other treatments. No differences were seen between the proportion of extractable copper within the UPS and APS materials.

By t=105 days, very little had changed within the sodium acetate extractable fraction of the unamended materials. Small decreases were seen for the UPS and UCS in the amounts and proportions of copper extracted but these may not have been significant. Decreases were seen however, within the AGS and ACS blends, the latter decreasing from 20.66% to only 2.40% of the proportion of extractable copper; while the APS saw a large increase of 224.61 mg kg^{-1}.

However, in terms of the percentage share, this amounted to an increase of no more than 4.55%. Neither the AGS nor the ACS differed from their controls in terms of either the amount or the proportions of copper extracted.

At the end of composting neither blend effects nor blend x treatment interactions were significant (p=0.100 each) for the amounts of copper extractable at this stage. Treatment effects were still very highly significant though (p≤0.001). All the materials, both copper treated and untreated, saw increases in the amounts of sodium acetate extractable copper during this second and final phase. However, few significant differences between the final extractable copper content in the materials were seen and even fewer in the extent to which any of the treatments had changed over the second phase.

c) Fraction 3 – Hydroxylamine Hydrochloride Extract – Reducible Fraction

For the amounts of extractable copper at t=0 days, all effects and interactions were very highly significant. The copper amended grass/sawdust blend contained significantly more copper (294.7 mg kg^{-1}) within the reducible fraction than any other treatment. No significant differences were found between any of the others. In terms of the percentage share, all the amended materials contained significantly greater proportions of extractable copper than the unamended, although the APS actually contained a smaller percentage of copper than the control.

At t=105 days all effects and interactions were still very highly significant ($p \leq 0.001$). No unamended material differed from another and all contained significantly less copper than the copper acetate treated materials. All blends and treatments, except the UGS material, saw an increase in the copper content of the reducible fraction. The APS now surpassed the AGS and contained 1333 mg kg^{-1}. It was clear from the data that most of the changes within this fraction occurred during these first 105 days.

Greater variation was seen amongst the percentage share data. With the exception of the AGS and the ACS, all the materials contained significantly different proportions of copper within the reducible fraction, with the control materials containing larger proportions than the copper amended materials.

At the end of the second phase of the composting process all effects and interactions were still very highly significant ($p \leq 0.001$). The unamended materials still contained significantly less copper than the copper acetate amended treatments. The AGS and ACS treatments were now no longer significantly different from each other in terms of the amounts which could be extracted. They were also not significantly differ in terms of the percentage share. The APS still contained significantly more copper than the other treatments but appeared to have decreased slightly when compared with the results from t=105 days.

The amount of change undergone by the different materials over this last stage of the composting process depended on the blend and treatment involved. The APS changed significantly more than the other treatments, increasing by 133.76 mg kg^{-1}. No significant differences were seen between any of the other treatments even though the behaviour seemed rather varied. Some materials appeared to increase (e.g. ACS) their copper content, while others decreased (e.g. UPS).

d) Fraction 4 – Oxidisable Fraction

At t=0 days all effects and interactions for the amounts of copper found within this fraction were very highly significant ($p \leq 0.001$). Copper within the oxidisable fraction in both the AGS and APS materials significantly exceeded those of the unamended materials, thus demonstrating that a significant amount of the additional copper was quickly converted to these forms. In contrast, no significant difference was seen between the unamended and copper treated sewage sludge cake/sawdust materials. Clearly, the additional copper in these materials had not been rapidly converted in the same manner. In terms of the proportion of copper held within this fraction all the treatments differed significantly from one another, with the unamended materials all containing the greater proportions of copper held.

After 105 days all the copper acetate amended materials contained significantly larger amounts of copper than the unamended. Less variation was seen between the unamended materials than at the start of the experiment and a significant difference was also seen between the amount of copper in the oxidisable fractions of the UCS and ACS materials.

In terms of the proportion of copper held within this fraction, all the unamended materials still contained within this fraction a significantly greater proportion of the total amount of copper available for extraction. Amongst these control materials only the UGS was significantly different from the UPS and amongst the amended materials the AGS contained a significantly smaller proportion than all the others, mainly due to the fact that a large proportion of the copper within the material was held within the magnesium chloride extractable and reducible fractions.

At the end of the experiment (t=318 days) all effects and interactions were still very highly significant. Copper acetate amended treatments still contained significantly larger quantities of copper than the unamended materials. However, blend effects for the percentage share data were no longer significant, though treatment effects were very highly significant ($p \leq 0.001$) and blend x treatment interactions were significant ($p=0.010$). The control materials still contained the largest proportions of the extractable copper and no control material differed significantly from another. Amongst the copper acetate amended materials only the ACS was significantly greater than the AGS blend.

With the exception of the APS, no blend or treatment differed in the extent to which it changed in the proportion of copper held within this fraction during this last phase. The pig slurry/sawdust was different in that it dramatically increased both the amount and proportion of copper held within the oxidisable fraction.

e) Fraction 5 – Residual Fraction

For many of the materials and treatments, at t=0 days, this fraction contained the least amount of copper compared with the other four extractable fractions. The APS was an exception in that it contained a large amount of residual copper (555.0 mg kg^{-1}/16.92% of the total extractable amount) – significantly more than all the other materials, including the UPS, thus clearly showing that some of the additional copper had been very quickly bound to the binding sites included in this fraction. No significant differences were seen between the unamended and the copper acetate treatments for the other materials' percentage share data. All effects and interactions for both the amounts of residual copper and proportions of the total extractable amount were found to be very highly significant ($p \leq 0.001$).

After 105 days into the composting process blend and treatment effects were still very highly significant ($p \leq 0.001$) and blend x treatment interactions highly significant ($p = 0.005$). All the control materials contained significantly less residual copper than their copper acetate amended counterparts and, while for some materials either no change or very small increases in the amounts of residual

copper were seen, the APS decreased quite substantially by over 480 mg kg^{-1}. Fewer variations compared with t=0 days were seen in the percentage share data and now the UCS was found to contain the greatest proportion of residual copper (4.19%).

By t=318 days all effects and interactions were very highly significant ($p \leq 0.001$) and no significant differences were seen between any of the control materials. The AGS and ACS blends contained more copper than the controls but did not differ significantly from each other. The APS still contained the largest amount of residual copper (256.6 mg kg^{-1}), an increase from the 73.96 mg kg^{-1}extracted at t=105 days. It was interesting to note that the AGS contained a greater proportion of residual copper than the UGS; the UCS contained a smaller proportion of residual copper than the unamended blend and no significant difference at all was found between the unamended and the APS.

During the second phase of the experiment, all blends, with the exception of the UGS, accumulated residual forms of copper. Few significant differences though were seen between the treatments and only the APS accumulated significantly more than all the other treatments. No significant differences were seen between any of the controls - all accumulated similar amounts of copper. Changes in the proportion of extractable copper found within this fraction were small. No significant differences were seen between the unamended and the copper acetate treatments in both the grass/sawdust and pig slurry/sawdust materials. A

significant difference was seen though between the ACS (increase of 4.75 percentage points) UCS (increase of 2.84 percentage points).

Over the entire experiment, all blends, except the UGS and the APS, saw a net increase in the amounts and proportions of copper found within their residual fractions, with the greatest accumulation being seen in the ACS. No change was observed in the UGS and the APS saw a net decrease in residual copper forms as the metal was released to other fractions.

f) Total Copper Extracted (Sum of Fractions 1-5)

Results for the total amounts of copper extracted for the various treatments can be found in Figure 3.8.

At t=0 days the total amount of copper extracted from all the materials ranged from 1.3 mg kg^{-1} to 3277 mg kg^{-1}.

Copper was added to the materials at a rate of 1000 mg Cu kg^{-1} materials on a fresh weight basis. Taking the moisture contents of the materials into account, this meant that it was added at rates of 1871 mg kg^{-1}, 2591 mg kg^{-1} and 2028 mg kg^{-1} on a dry matter basis for the grass/sawdust, pig slurry/sawdust and sewage sludge cake/sawdust materials respectively. Variation within the data for the

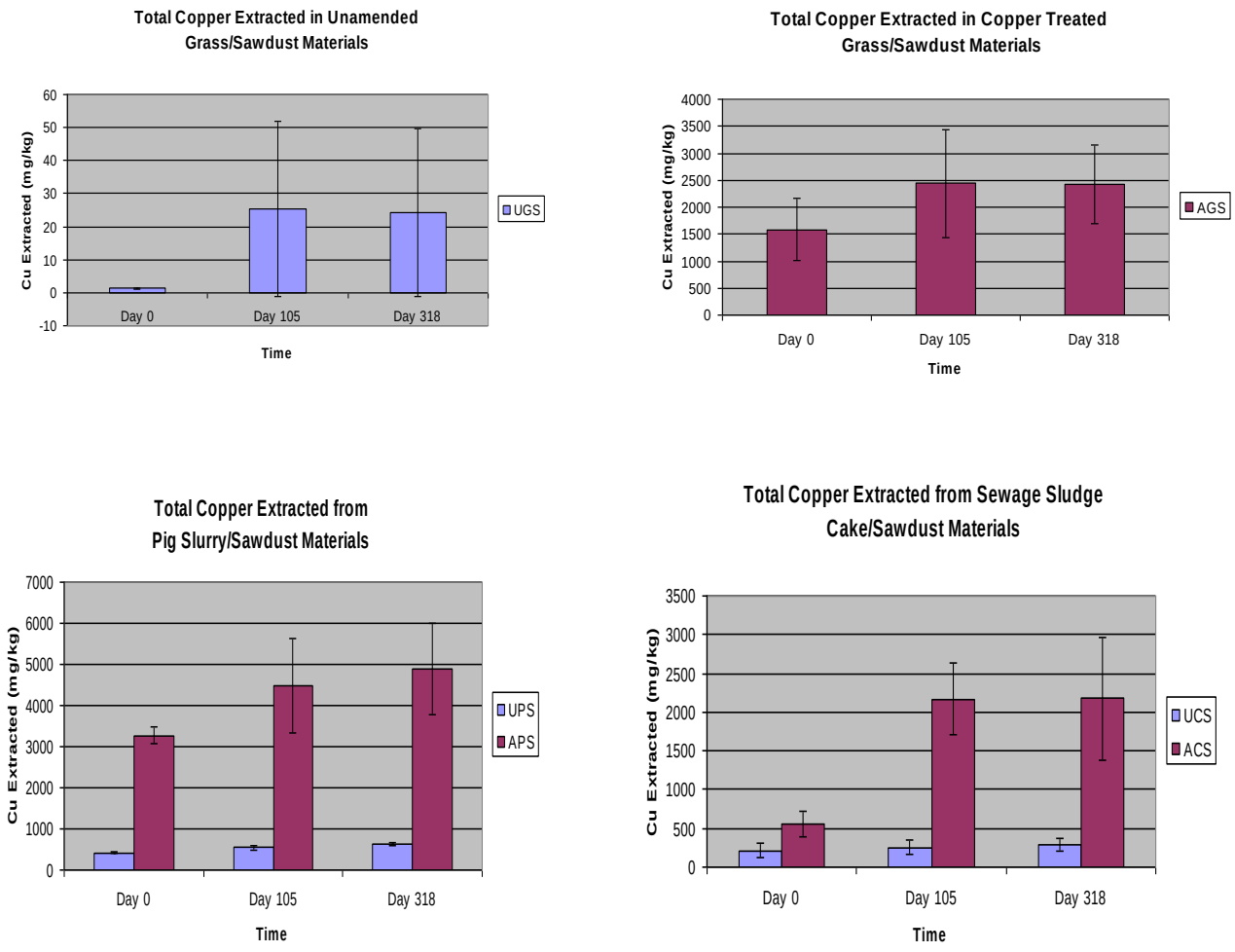


Fig. 3.8 Total Copper (Sum of 5 Fractions) Extracted from All Unamended and Copper Acetate Treated Materials by the Tessier et al (1979) Sequential Extraction Scheme. Error bars are ± 95% Confidence Intervals

UGS, AGS, UPS and APS meant that recoveries were not statistically different from the calculated application rates. The copper amended sewage sludge cake materials (ACS) were very different though. Only 552 mg Cu kg^{-1} were removed during the extraction. As this figure was significantly different from that of the UCS (it was more than double that of the unamended materials), it was concluded that this material had actually received an application of copper acetate. However, total copper as determined by XRF (See Section 3.4) was even lower (208 mg kg^{-1}). Some of each of the materials prepared for composting were set aside for analysis before the rest were added to the composting units. As copper extracted from the ACS at t =105 days was a realistic 2166 mg kg^{-1} it must therefore be concluded that the low recovery of copper in this instance was due to the materials set aside at t= 0 having been poorly sampled and was not due to problems with the sequential extraction scheme.

All effects and interactions were very highly significant; less copper had been extracted from the control materials than from the copper acetate treatments; all the copper amended materials were significantly different from each other, while among the controls, less variation was seen, the only significant difference being between the UGS and the UPS.

By the end of the first phase of the experiment there were still no significant differences in the total amount of copper that had been extracted from the control materials. The copper acetate treatments were still significantly different from the

controls but now there was no difference seen between the grass/sawdust and sewage sludge cake/sawdust materials. The APS continued to contain significantly larger amounts of extractable copper. The various differences seen among the treatments at t=105 days remained unchanged throughout the second phase of the experiment and had not altered by t=318 days. No measurable differences were seen in the behaviour of any of the treatments during the second part of the experiment, except in the APS. This increased by 415 mg kg^{-1}, significantly more than all the other materials. The range for all the other materials was from a decrease of 25.52 mg kg^{-1} (AGS) to an increase of 84.34 mg kg^{-1} (UPS). Most of the changes in the total extractable amounts of copper appeared to occurr during the first phase of the experiment and only very small changes seemed to occurr during the second. However, none or these were significant at 95% confidence intervals. Lack of significant differences may be due to large amounts of variation within the data, as exemplified by the very high CV value (103.1%) and greater replication may have revealed more differences than was able to be shown here. Nevertheless, as no significant changes were seen in the materials' organic matter contents (Section 3.6) either, this may be due more to the lack of degradation of the materials.

Regression Analysis

Regression analysis of the sequential extraction data was carried out and it has been found that it is possible to model the behaviour of easily extractable forms

of copper and predict the likely time required for copper in this range of materials (which are high in $MgCl_2$ extractable copper) to be rendered unavailable to plants by the composting process. Regression analyses were performed in an attempt to model the behaviour of the copper and thus predict the likely behaviour of future composting systems (Fig. 3.9).

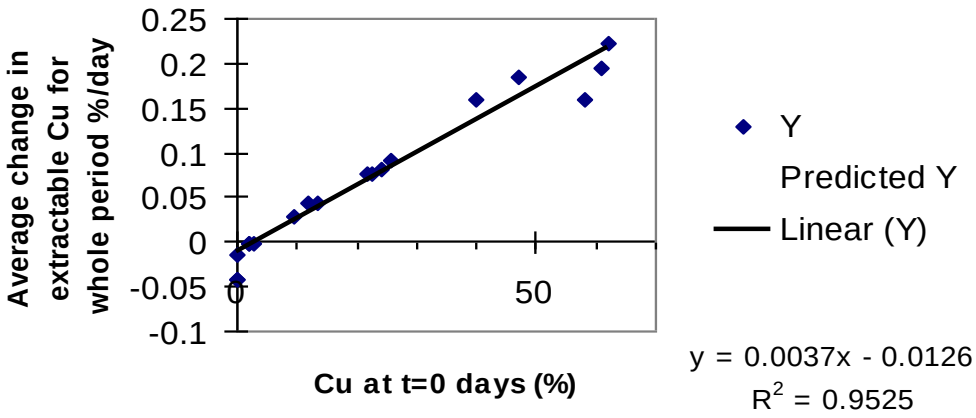


Fig. 3.9 Regression Analysis of Change in $MgCl_2$ Extractable Copper Over the Whole Experiment Against Initial Extractable Copper Levels as a Percentage of the Total Extractable Amount.

Extractants were considered individually and the magnitude of change of extractable copper in mg Cu per day either over the whole experiment or over just one phase of it was plotted against the initial amount of copper extracted by that reagent (or set of reagents in the case of the oxidisable and residual fractions). Data from each replicate for all the different materials composted were used for these regressions. The best regression was achieved with changes in

$MgCl_2$ extractable copper over the entire composting period plotted against initial extracted copper (Fig. 3.9). The R^2 value for this was 0.95.

3.2.3.3 Discussion

A: Grass/Sawdust Blends – All Treatments

Changes in the chemical form of copper were seen to occur in all treatments of this blend throughout the course of the composting period. This was as a result of the process itself.

Unamended grass/sawdust materials:

This blend behaved very differently to the copper amended materials and is therefore discussed here separately. Unlike the material composted in Trial 1, all the copper in this material was found to be present in Fraction 4, the oxidisable fraction, which extracts metals bound within the organic fraction of the material. In the starting materials of Compost Trial 1, over 80% of the copper was found in residual forms and the total amount present exceeded 490 mg kg^{-1}. These large amounts were attributed to the fact that the sawdust had come from tanalised timber. In Compost Trial 2 the sawdust originated from non-tanalised timber and so had very different characteristics and behaviour.

At t=0 days the sum total of extractable copper was just 1.3 mg kg^{-1}, all of which was removed in step 4, the oxidisable fraction. Over the course of the experiment the proportion of copper held within this fraction decreased to 90.34% of the total extractable amount, with corresponding increases, first in the magnesium chloride extractable fraction and then, during the second phase of the process, small increases in the sodium acetate and reducible fractions. The majority of changes occurred during the first 105 days (which included the thermophilic phase) although oxidisable forms dominated throughout the course of the composting period.

Copper is consistently found to be predominantly extracted by those reagents believed to extract organically associated forms (Gao *et. al.,* 2005; Ciba *et. al.;* 1999; Chwastowska and Sterlinska, 2000; Chwastowska and Skalmowski, 1997; Tisdell and Breslin, 1995; Bourque *et. al.,* 1994;), often accounting for up to 90% of the copper present in the material (Chwastowska and Skalmowski, 1997). Such reports are nevertheless largely restricted to the analysis of uncharacterised municipal solid waste (MSW) and do not specify the composting of green wastes. However, Greenway and Song (2002b) studied the composting of various green wastes and found that copper was largely associated with the organic fraction. Copper was also extracted during earlier steps of the sequential extraction and decreases over time were noted, something which did not occur during this trial.

Decreases in organic forms have also been the experience of other workers who found that this was attributable to pH changes due to the formation of acetic acid during organic matter breakdown and changes in ammonia content due to the action of the proteolytic bacteria and by the high temperatures. These conditions would also produces oxic and anoxic conditions which affect the reducible and organic fractions (Zorpas *et al.*, 2000).

It is difficult to draw comparisons between the results found here and those of others as no other examples were found in the literature which covered the changes in the distribution of native copper during the composting of these kinds of materials.

Copper Acetate, Copper Sulphate and Copper EDTA Treated Grass/Sawdust Blended Materials

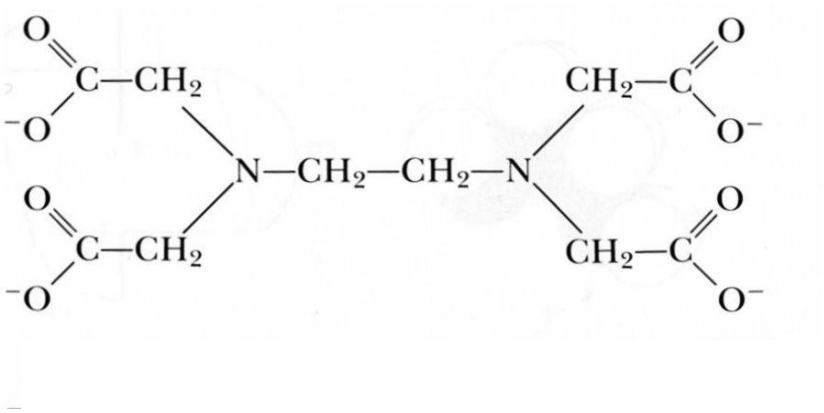
In all three of the copper amended treatments substantial changes were seen in both the magnesium chloride extractable and the 'organic' (oxidisable) fractions and smaller increases in the sodium acetate extractable and the reducible fractions.

Copper distribution amongst the different fractions, and its changes over time did, however, depend on the chemical form in which it was originally applied. The AGS and SGS treatments behaved in very similar ways often with no significant

differences between them in the proportions of copper in their various fractions at any of the sampling times. The trend was for copper to move from the easily available fractions, especially the magnesium chloride extractable fraction, to less easily available fractions (particularly the organic fraction), followed by the reducible fraction and then the sodium acetate extractable fraction. It would appear that copper in the SGS treatment moved from the magnesium chloride fraction more slowly during the thermophilic phase than in the AGS. However, by the end of the experiment both treatments held proportionately the same copper in all the different fractions, just as they did at the start. Copper (II) salts, such as copper acetate and sulphate, dissociate readily in water to form hydrated Cu (II) complexes (Atkins, 1989). These will then readily combine with the organic ligands which emerge during composting. The chemical reactions which then take place between the copper (II) complexes and the various ligands present in the materials are therefore likely to be similar in both cases.

Changes in the distribution of copper in the EGS were a little different. The patterns of distribution were similar but the speed at which changes occurred was substantially different. At t=0 days 93.65% of the copper present was as magnesium chloride extractable copper, approximately 1/3 more than with

A



B

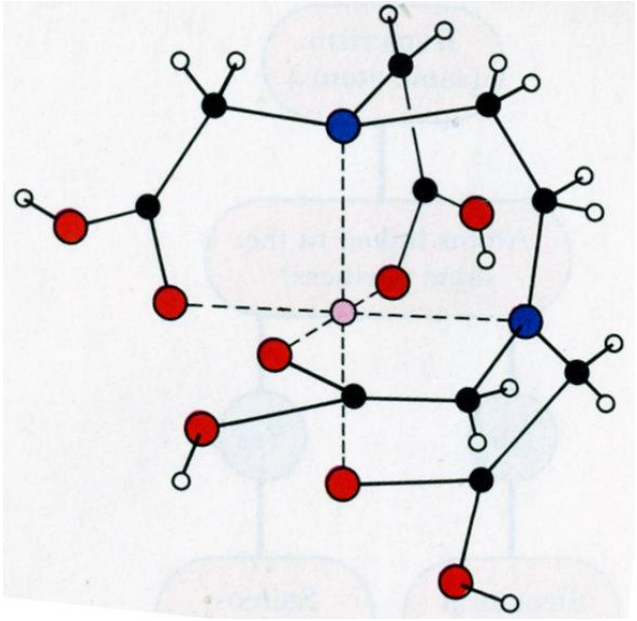


Fig. 3.10 A: Ethylenediaminetetraaceto ligand and **B**: EDTA forming a 6:1 coordinated chelate with a metal (Atkins, 1989).

the copper acetate and sulphate treatments. Small amounts were also extracted from the sodium acetate extractable, organic and residual fractions but these were much smaller, both in size and proportion, than in the copper acetate and copper sulphate treatments. They still exceeded the amounts extracted from the

unamended grass/sawdust blend, so it is possible to conclude that even at t=0 days there were still some available ligands which could effectively compete with the EDTA for the copper.

During the first phase, copper in the EGS material moved from the easily available magnesium chloride extractable fraction, to the organic and reducible fractions. During the second phase, copper in these fractions continued to increase and copper also moved from the magnesium chloride to the sodium acetate extractable fraction, so that by t=318 days, the proportion of copper in the various fractions of the EGS was similar to those of the AGS and SGS treatments. Only in the magnesium chloride and reducible fractions were there significant differences – the EGS treatment contained a significantly greater proportion of copper in its magnesium chloride fraction and a significantly smaller proportion in the reducible fraction the other copper treatments. It is hypothesised that, given a longer period of time for composting, all three copper treatments would contain identical proportions of copper in their five extracted fractions. This difference in rate of change can be explained by the fact that the copper ion is bound to the EDTA molecule far more strongly than it is associated with the sulphate and carboxylate anions. EDTA (Fig. 3.10A) is a hexadentate chelating ligand and its two N atoms and four of its O atoms can occupy all six octahedral bonding positions around a metal atom, gripping it in a single six-pronged claw or chelate structure (Fig. 3.10B). Copper, which acts as a Lewis acid and is able to bind with both oxygen and nitrogen lone pairs of electrons, is thus very tightly

bound up in this structure. The time taken for it to bind, associate or complex with other ligands present in the composting material will therefore by necessity take considerably longer to achieve; either because of the time taken to produce ligands which can effectively compete with the EDTA or, possibly, because of the greater energy requirements of bacteria to break apart the chelation complex. It is therefore likely that within the samples taken at t=0 days, most of the copper EDTA solution merely coated the sample particles and was therefore easily washed off in the magnesium chloride solution.

Residual forms of copper play only a very small part in the overall distribution of copper within all the treatments of this blend. In the environment, metals in this fraction are not considered as being available for plant uptake. So, whilst their role would be important, the fact that the proportion of copper held here is so small means that their significance is tiny.

No work of this kind which studies the behaviour of metals within composting materials artificially contaminated with pure chemicals has previously been reported. It is not therefore possible to compare these results with those of other workers.

<u>B: All Blends – Unamended and Copper Acetate Treatments</u>

Copper in the UGS and AGS has already been discussed in detail in the last section. It is therefore only considered here for comparison with the brown waste blends.

Pig Slurry/Sawdust Blend – Unamended and Copper Acetate Treated Materials

The vast majority of copper in the unamended material was held within the oxidisable (organic matter) fraction at the start of the experiment. During the first phase of the composting period copper was released from this fraction primarily to the reducible and the magnesium chloride and sodium acetate extractable fractions. It is here, at the end of the first phase, that the 'readily available' copper was at its peak. During the second phase, some of this copper was redistributed, with some returning to the oxidisable fraction and some to the residual, the amount of which more than doubled. Most of the copper though was still within the oxidisable fraction (>86%). Clearly, some of the copper was becoming more tightly bound as the composting process proceeded. However, alongside this there was a definite net increase in the proportion of 'readily available' copper i.e. magnesium chloride and sodium acetate extractable copper combined. The two fractions together thus gave a total of 22.8 mg kg^{-1}, a net increase of 10.26 mg kg^{-1} of 'readily available' copper, almost double that available at the start. These findings support those of Liu *et. al.* (2003) who analysed co-composted pig manure with sawdust and coffee dregs and found that more than 95% of the copper was present in the organic fraction (they did not measure residual forms).

Hsu and Lo (2001) who, using the scheme of He *et. al.*(1995) studied the composting of separated swine manure and found that copper was associated strongly with the so-called 'organically bound' or 'oxidisable' fraction of the scheme. They also discovered that water soluble copper increased during the first 18 days of composting and that these increases corresponded with decreases in copper found in the organically bound and organically complexed fractions.

Huang *et. al.* (2005) co-composted pig manure with sawdust at a ratio of 4:1 to obtain a C/N ratio of around 30 and having an overall copper content at the start of the process of 556 mg kg^{-1}. These workers obtained different results to those found in this trial. They found that copper was largely associated with the organic and residual fractions both at the start of composting and at the end (only 63 days later); that the percentages of water extractable, exchangeable, carbonate and Fe-Mn oxide forms of copper decreased significantly during composting; the organic fraction of copper increased from 32.7% to 41.6%; residual copper increased from 29.9% to 40% and by the end of their experiment total copper had actually decreased to 78 mg kg^{-1}, which they attributed to the diluting effect of the sawdust. This last point does not make sense. Sawdust was included at the start of their trial and, even if there was only a small reduction in the volume of materials over those 63 days, total copper content should have increased. Total copper content at the start was similar to that found in this trial for the unamended pig slurry/sawdust material (418 mg kg^{-1}). However, the percentages

of copper in their organic fraction was much lower (32.7% at the start compared with 91.31% for the UPS) and the percentage of copper in their residual fraction was much higher (29.9% at the start compared with 2.55%). It must therefore be concluded that their pig manure and sawdust is of a very different nature and that perhaps their sawdust came from tanalised timber.

The APS exhibited a marked decrease in both magnesium chloride extractable and 'residual' copper with corresponding increases in sodium acetate extractable and copper held within the reducible fraction. A small decrease in the proportion of recoverable copper held by the 'oxidisable' fraction was observed over the first phase of the experiment. However, the actual quantity held increased by around 700 mg kg^{-1} though this was a smaller increase than that of the total recoverable copper which suggested that a transformation into other forms had taken place. The percentage of copper in the organic fraction increased over the second phase of the experiment owing to decreases in both residual and magnesium chloride extractable forms. Clearly, the additional copper was transformed from easily available to less available forms as the compost matured.

Sewage Sludge Cake/Sawdust Blend – Unamended and Copper Acetate Treated Materials.

The vast majority of the copper was contained within the 'oxidisable' fraction for both the treated (ACS) and untreated (UCS) sewage sludge cake blends

throughout the course of the experiment. At t=0 days around 11% of the copper extracted from the untreated blend was 'readily available' as $MgCl_2$ extractable – approximately 25 mg kg^{-1} - but this decreased over time to around 2.6% by the end of the experiment (around 7.6 mg kg^{-1}). Transfer of copper to less available forms appears to entail conversion of copper from primarily the magnesium chloride and sodium acetate extractable forms during the thermophilic phase to forms held within the reducible and oxidisable fractions at a ratio of around 3:1 reducible fraction to oxidisable fraction forms. During the second phase of the experiment, magnesium chloride extractable forms of copper continued to decrease. Copper within the reducible fraction decreased too. These decreases led to the formation of residual, organic and some sodium acetate extractable forms, though changes in the latter may not actually be significant. Thus, during composting, some of the copper redistribution is seen to occur via the reducible fraction, as can be seen by its slight increase and then decrease over the course of the process and also the corresponding increases in the amounts held in both the oxidisable and residual fractions.

The pattern of redistribution is similar in the copper acetate treatment. During the first phase copper is removed primarily from the $MgCl_2$ extractable fraction, secondarily the NaOAc extractable fraction, and to a much lesser extent, the residual fraction. It is transferred primarily to the 'oxidisable' fraction (which increases by 36% of the total extractable amount; 1120 mg kg^{-1} over the first phase of the process) and secondarily to the 'reducible fraction' (increase of

10.5% of the total extractable amount; 400 mg kg^{-1}, also over the first phase). The slight fall in the residual fraction amounts to only 0.7 percentage points during this period. However, the amount which is extractable rises from 16 to 48 mg kg^{-1}. So, although the amount has more than doubled, the proportion held remains virtually the same, suggesting this increase is likely to be a result of the concentrating effect of the composting process rather than an actual change in the chemical form itself.

During the second half of the process magnesium chloride extractable forms continue to decrease, leading to increases in the proportions held in all other fractions. The rise and fall in the reducible fraction that was seen in the UCS was not seen here in the ACS. Reducible forms do not therefore appear to play the same kind of 'stepping stone' role they did before. The 'residual' fraction more than doubled the proportion of copper that it held, increasing from 4.18% of the total to 8.9%. The amount held also more than doubled, increasing from 48 mg kg^{-1} to 110 mg kg^{-1}. The sum of all the fractions remained the same too over this second phase. Thus it can be said that these increases are solely as a result of changes in the chemical form of the copper and not as a result of further losses in volume of the composting material.

Reports in the literature of the sequential analyses of sewage sludge cake compost are limited. Results of these analyses vary somewhat, but what is clear is that changes in the behaviour of organic, residual and easily

exchangeable/extractable forms of copper dominate the patterns of redistribution and as no examples include the addition of any form of copper solution it is appropriate to compare the results of these reports with those of the untreated sewage sludge cake/sawdust material. Most reports in the literature declare that organic forms of copper dominate at the start of composting (Gao *et. al.* 2005; Liu *et. al.* 2003; Zorpas *et. al.* 2003 and 2000; Paré *et. al.* 1999; Qiao and Ho 1997) but then redistribute over time – mainly to the residual fraction. Organic forms may still dominate in the end product but they were found to substantially decrease. This is in contrast to the sewage sludge cake/sawdust materials used in this trial. Here the proportion of organically bound forms of copper dominated but also continued to increase. In some reported cases where organic copper was seen to dominate, residual forms of copper at the start of composting were found to be the next most dominant form of copper (Gao *et. al.* 2005; Zorpas *et. al.* 2003 and 2000) which then increased substantially during the composting process. However, residual forms of copper played a much smaller role in the composting of the UCS materials. Magnesium chloride extractable forms were the second most dominant form with residual forms following. Magnesium chloride and sodium acetate extractable forms were found to substantially decrease over time, which supports the findings of others in that copper extracted in the earlier stages of the scheme were seen to decrease with composting time (Gao *et. al.* 2005; Amir *et. al.* 2005 and Planquart *et. al.* 1999).

There is not a complete consensus of opinion within the literature regarding the behaviour of sequentially extracted copper from sewage sludge cake composts. In contrast to the findings of those already mentioned, other workers have found that residual and not organic forms dominate (Amir *et. al.* 2005; Planquart *et. al.* 1999), sometimes increasing further (Planquart *et. al.* 1999) with time; and that occasionally the easily extractable copper forms increase over time too (Zorpas *et. al.* 2003 and 2000; Qiao and Ho, 1997). As one author pointed out (Planquart *et. al.* 1999), sewage sludge composts 'do not have similar metal distribution according to their origin and the treatment undergone and hence, the speciation of trace metals must be considered for each compost on an individual basis in order to predict their behaviour…' This has certainly proved to be true for all the samples obtained in this trial, not just those produced from sewage sludge cake.

Changes in copper distribution within sewage sludge composts have been associated or correlated with changes in various parameters such as pH (Gao *et. al.* 2005), conductivity (Zorpas *et. al.* 2003;) and functional groups (Amir *et. al.* 2005). Measurements of these parameters were also carried out on the samples obtained from this second compost trial and will be discussed in detail in a later section.

Regression Analysis

The best regression was achieved with changes in $MgCl_2$ extractable copper over the entire composting period plotted against initial extracted copper (Fig. 3.9). The R^2 value for this was 0.95.

It is extremely interesting to note that blend type appears only to influence the initial amounts of extractable copper. It is then the initial amount itself that directly determines the magnitude of change during the composting process. This provides a functional assessment of speciation and goes some considerable way to predicting the length of time required for materials to compost before they can be declared 'safe' to grow plants in. It seems that this may be done with reasonable certainty once the initial phase of composting is complete. This is particularly important in light of the fact that magnesium chloride extractable copper is regarded as being that which is most likely to be available for plant uptake.

3.2.3.4. Summary of Key Findings

- Changes in copper distribution were seen in all blends, for all treatments, throughout the composting process
- Copper originally present in all blends was predominantly found within the organic or 'oxidisable' fraction.

- Approximately 10% of copper original to the grass/sawdust blend was released during the first composting phase to more easily extractable fractions.
- Most of the additional copper added to all three of the composting blends was initially found in the magnesium chloride extractable fraction but then moved to less easily extractable fractions.
- Copper added as copper sulphate and copper acetate to the grass/sawdust blend redistributed in similar ways and at similar rates.
- Copper added as copper EDTA to the grass/sawdust blend redistributed at a much slower rate owing to the manner in which copper is bound to the EDTA ligand.
- Copper originally present in the pig slurry/sawdust blend was released from the oxidisable fraction to more easily extractable and reducible fractions during the first phase but then some was redistributed back to less available fractions during the second phase.
- Redistribution of copper in the APS occurred via the reducible fraction.
- Approximately 11% of copper original to the pig slurry/sawdust blend at t=0 days was magnesium chloride extractable. This decreased over time and was converted to organically complexed forms (held within the oxidisable fraction) via the reducible fraction.
- Movement of copper from the magnesium chloride extractable fraction to the oxidisable fraction also occurred in the ACS but not via the reducible fraction.

- Regression analysis showed that blend type influenced the initial amounts of magnesium chloride extractable copper. This initial amount of magnesium chloride extractable copper then determined the magnitude of change in this fraction during composting.

3.3 Chelating Resin Membrane Analysis

Phytoavailable copper in the samples obtained during this trial was measured using a diffusion sensitive system. Samples were wetted to field capacity and strips of chelating resin membrane were buried within them for 24 hours, ensuring good contact between the material and the resin surface. The principle behind this approach is that copper in the moisture in the material immediately adjacent to the membrane chelates with the diethylenetriamine pentaacetic acid (DTPA) on the exchange sites of the resin membrane (which acts as a sink for the metal) and is thus removed from the material. A gradient is then established, causing copper at a slightly greater distance away from the strip to diffuse towards the membrane strips. This copper also chelates with the DTPA and is removed from the compost. In the absence of other competing sinks or metals the process continues until either the membrane is saturated or the strips are removed from the material. Some workers have chosen to use saturated soil pastes (Yang *et. al.,* 1991a) when employing diffusion sensitive systems. However, field capacity - a term normally used in connection with soils - was chosen because it is used as a marker of the upper level of the water content at which water is ordinarily available for plants (Marshall and Holmes, 1988). It was therefore felt to be a more appropriate condition in which to have the samples, the trial being an attempt at establishing the likely phytoavailability of the copper.

3.3.1 Materials and Methods

Determination of Field Capacity

The field capacities (FC) of the trial samples were calculated from the water required to bring to FC samples of the sawdust used in the trial, and two commercially available composts (a multipurpose and a seed compost). Regression analysis was carried out on the water required for these materials and the slope obtained enabled FC values of the trial samples themselves to be calculated. Direct measurements of the FC values of the samples themselves were not possible owing to the lack of sample material. It was assumed that the FC of samples taken at t = 0 days would closely resemble that of the sawdust (sawdust being the dominant fraction), while values for samples taken at t = 105 and 318 days would more closely resemble those of the multipurpose and seed composts. Field capacities of the sawdust and the commercially available composts were determined according to the method of Inbar *et. al.* (1986). This involved the construction of cylindrical lysimeters, of height 10.8cm x diameter 7.3cm with 2 layers of muslin secured to the bases with twine and tape and their weights recorded. Both composts and sawdust were air-dried for 3 days and their changes in weight calculated in terms of g H_2O/g compost. These air-dried materials were then pre-saturated for 48 hours with deionised water and then weighed into the lysimeters (in triplicate) to a depth of 10cm and left to drain for 2 hours. After draining, the lysimeters were reweighed and then oven-dried at 80°C

for 72 hours. Water lost during oven drying, expressed as g H_2O/g of air-dried material was taken to be the FC of the material.

To determine the amount of water required to bring the fresh compost samples to FC, small sub-samples were weighed into crucibles and left for 3 days to air-dry. Change in weight was then calculated in terms of g H_2O/g compost. From this point, using the values determined in the lysimeter experiment it was then possible to calculate the additional water required to bring fresh material up to FC.

Preparation of the Chelating Resin Membrane (CRM)

Anion-exchange membrane sheets (BDH product code 55164) measuring 12cm x 12cm were prepared according to the method of Liang and Schoenau (1995). Sheets were cut into strips measuring 6cm x 2cm and soaked for 24 hours in 0.01M DTPA + 0.02M NaOH to saturate the functional groups on the membrane with DTPA. After saturation, the strips were washed three times in deionised water in readiness for burial in the composted materials.

Resin Membrane Burial

Fresh samples were weighed into thin plastic bags measuring 23 x 19cm and the required amounts of deionised water added to bring the material up to its

calculated FC. Within the bags, approximately half the material was pressed flat to make a bed large enough on which to place the prepared resin membrane strips. Wherever possible, enough material was used to be able to bury 3 strips. However, sufficient material was not always available and sometimes only 2 strips could be used (and, very occasionally, only 1 strip). The remainder of the material was then scooped carefully over the top of the strips and pressed flat, ensuring that all the strips were completely covered over. The edges of the bag were then carefully folded over to prevent moisture loss and the created 'parcels' were left for 24 hours at room temperature for the copper in the compost to be chelated with the DTPA on the binding sites of the membrane. After incubation was complete, the membrane strips were carefully removed, rinsed with deionised water to remove any adhering particles and stored in a ziplock bag at 4°C until they could be analysed for adsorbed copper.

For the determination of adsorbed copper, individual membrane strips were placed in 50ml polyethylene tubes (Sarstedt) and shaken with 20ml of 0.1M HCl for 2 hours to elute the copper. Copper in the eluate was determined was by (AAS) at 324.7nm on a Smith Heiftje 1000 Atomic Absorption Spectrophotometer using matrix matched standards. To correct for interferences within the air-acetylene flame, which arose as a result of the presence of other extracted substances, 1 eluate from each blend, treatment type and sampling time was analysed for copper by AAS using standard additions in conjunction with the

matrix matched standards. Any differences in results were used to correct the results for the whole of that group of samples.

To check the total chelating capacity of the CRM strips, two of the DTPA prepared strips of membrane were set aside and soaked in 10,000 mgl^{-1} of $CuCl_2.2H_2O$ for 24 hours to saturate the DTPA sites with copper. The strips were then shaken with 0.1M HCl and the eluted copper determined in the same manner. Such an approach was recommended by Qian and Schoenau (1997), who advised against relying on manufacturer's reported exchange capacities and suggested saturating the membrane in the ion of interest, with subsequent elution and determination, as a convenient way of assessing the resin capacity.

Data for the UGS, AGS, SGS and EGS samples were analysed separately as one group (Group A) and the unamended and copper acetate treatments of the grass/sawdust; pig slurry/sawdust and sewage sludge cake/sawdust samples were analysed as another (Group B). As the data were not found to be normally distributed the results were first subjected to a $\log_{10}$ transformation before being subjected to statistical analysis. Transformed data were analysed fraction by fraction by Analysis of Variance (ANOVA) using Tukey's method of multiple comparisons between means at the 95% confidence interval and the software program Genstat® (8^{th} and 9^{th} Editions) was used. Transformed data for all samples were also correlated with the amounts of copper extracted within each

fraction (also $\log_{10}$ transformed) of the Tessier sequential extraction scheme using Microsoft Office Excel software

3.3.2 Results

Determination of Field Capacity of the Samples

Regression of the data was successful, giving a value of R^2 of 0.9997 (Fig. 3.11).

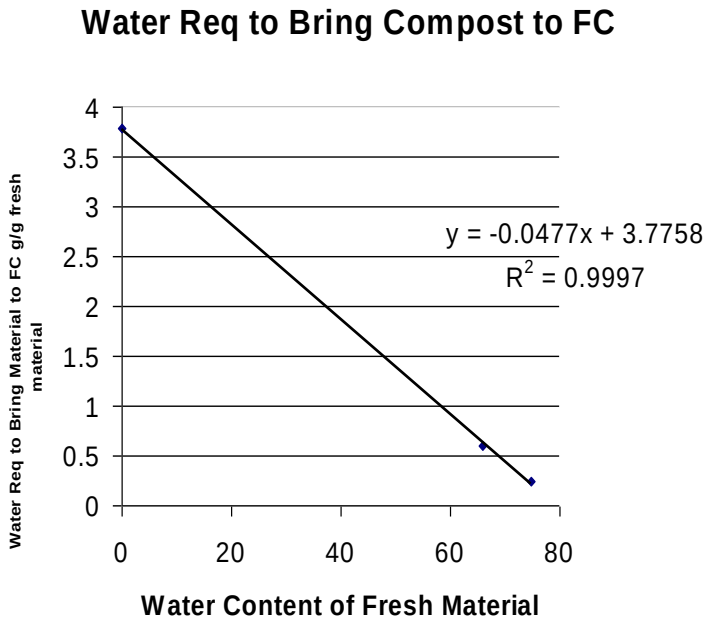


Fig. 3.11 Regression analysis of the Water Required to Bring to Field Capacity, Sawdust and 2 Commercially Available Composts.

From this equation, the water required for the trial samples to be brought to FC could be calculated.

Adsorptive Capacity of Membrane Strips

The CRM strips which had been soaked in the copper chloride solution for 24 hours gave a figure for the total copper chelating capacity of the membrane of just 2.96 ± 0.12 µg cm^{-2} (± 1 s.e.). This was much smaller than many of the results obtained with the resin membrane burials. Possible reasons for why this was the case are discussed later in the text.

Resin Membrane Burial

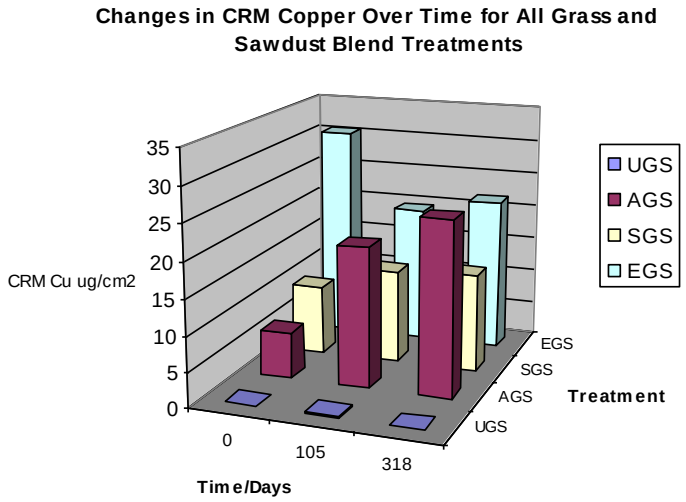
Group A: Grass/Sawdust Blend - Unamended, Copper Acetate, Copper Sulphate and Copper EDTA Treatments

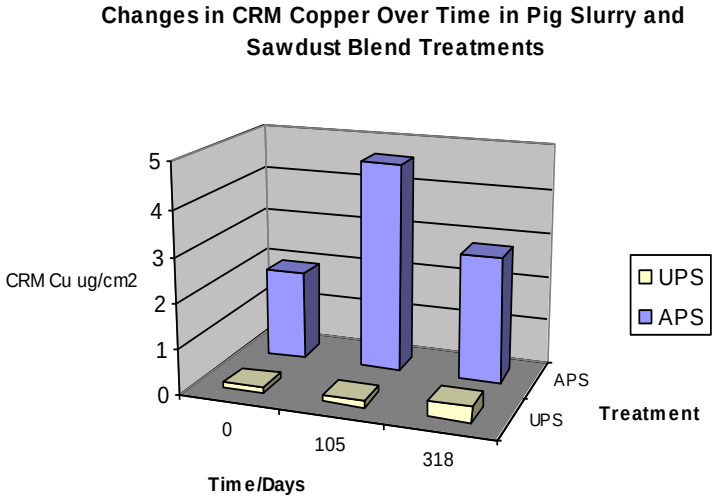
The data are presented in µg Cu cm^{-2} membrane (Table 3.24 and Fig. 3.12).

Table 3.24 Copper Adsorbed by Chelating Resin Membrane Strips from the Grass/Sawdust Blends. Transformed data are in parentheses for comparative purposes. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Compost Blend	T = 0 Days		T = 105 Days		T = 318 Days	
	Cu Extracted (µg cm^{-2})	%CV	Cu Extracted (µg cm^{-2})	%CV*	Cu Extracted (µg cm^{-2})	%CV*
UGS	0.135 (-0.872)	6.83	0.237 (-0.664)	3.90 - 26.06	0.054 (-1.269)	7.59 - 39.45
AGS	6.570 (0.814)	15.94	19.93 (1.129)[a]	5.50 - 12.00	24.67 (1.390)[a]	2.19 - 7.53
SGS	9.899 (0.994)	9.27	13.38 (1.102)[a]	0.00 - 6.54	14.07 (1.148)	2.91 - 4.79
EGS	30.63 (1.486)	3.93	19.65 (1.280)[a]	7.83 - 26.67	21.76 (1.336)[a]	7.09 - 8.50

* Values quoted are for the range across the 3 blocks. %CV = (standard deviation/mean)*100





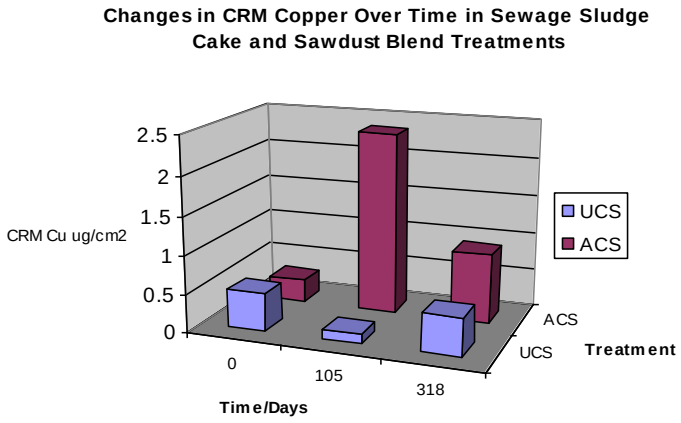


Fig. 3.12 Changes Over Time in Chelating Resin Membrane Extractable Copper Within Unamended and Copper Acetate Treated Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Materials

At the start of the experiment, treatment effects for CRM extractable copper were very highly significant ($p \leq 0.001$). All treatments contained significantly different amounts of extractable copper, with the copper EDTA treatment containing most of all. By the end of the first phase of the experiment, treatment effects were still very highly significant ($p \leq 0.001$) but less variation was seen. Large increases in CRM extractable copper were seen over this first phase in both the AGS and SGS treatments, whereas a substantial decrease was seen in the EGS such that by t = 105 days none of the copper amended treatments contained significantly different amounts of extractable copper. All, however, contained significantly more than the UGS which had changed very little in comparison. CRM extractable copper in all three copper treatments appeared to increase over the final phase of the experiment. However, as these changes were small (around 2 μg cm^{-2}) they may not have been significant.

Group B: Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends – Unamended and Copper Acetate Amended Treatments.

The data are presented in μg Cu cm^{-2} membrane (Table 3.25 and Fig 3.12). At t = 0 days, all effects and interactions were very highly significant Neither the UGS (0.135 μg cm^{-1}) nor the UPS (0.119 μg cm^{-1}) contained significantly different amounts of CRM extractable copper. The UCS did however, contain almost four times as much (0.498 μg cm^{-1}) as the other unamended treatments and even contained more than the ACS. All other blends were significantly different from

each other, with amended treatments containing extractable copper in the order: AGS (6.57 µg cm^{-1}) > APS (1.975 µg cm^{-1}) > ACS > (0.301 µg cm^{-1}).

Table 3.25 Copper Adsorbed by Chelating Resin Membrane Strips from the Grass/Sawdust; Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends. Transformed data are in parentheses for comparative purposes. Identical superscript lettering within each column signifies no significant differences between pairs of results.

Compost Blend	T = 0 Days		T = 105 Days		T = 318 Days	
	Cu Extracted (µg cm^{-2})	%CV	Cu Extracted (µg cm^{-2})	%CV*	Cu Extracted (µg cm^{-2})	%CV*
UGS	0.135 (-0.872)[a]	6.83	0.237 (-0.664)[a]	3.90 - 26.06	0.054 (-1.269)	7.59 - 39.45
AGS	6.570 (0.814)	15.94	19.93 (1.129)	5.50 -12.00	24.67 (1.390)	2.19 - 7.53
UPS	0.119 (-0.926)[a]	3.72	0.134 (-0.873)[a]	14.67 - 25.73	0.330 (-0.485)[a]	9.18 - 26.94
APS	1.975 (0.296)	1.56	4.644 (0.635)[b]	15.03 - 56.20	2.791 (0.438)	7.98 - 22.25
UCS	0.498 (-0.304)	9.84	0.118 (-0.936)[a]	0.08 - 23.73	0.485 (-0.314)[a]	4.58 - 61.90
ACS	0.301 (-0.528)	21.97	2.359 (0.3534)[b]	1.76 - 15.96	0.900 (-0.049)	22.60 - 32.41

* Values quoted are for the range across the 3 blocks.

After the first phase of the experiment (t = 105 days), blend and treatment effects were very highly significant ($p \leq 0.001$) and blend x treatment interactions were significant ($p = 0.019$). None of the three unamended treatments were significantly different from each other, the UCS appearing to have decreased in its CRM extractable copper during this phase. Small, apparent increases in the CRM extractable copper content of the other two unamended treatments may not have been significant. All copper acetate amended blends seemed to increase their CRM extractable copper content during this phase and all three contained

significantly more CRM extractable copper than the controls. Relationships between the amended treatments remained similar and of the apparent order: AGS (19.93 µg cm^{-1}) > APS (4.644 µg cm^{-1}) > ACS (2.359 µg cm^{-1}), although it must be noted that there was no actual significant difference found between the APS and the ACS blends.

All effects and interactions were very highly significant ($p \leq 0.001$) by t = 318 days. A decrease in CRM extractable copper in the UGS during this last phase meant that the end product contained significantly less than the other two unamended treatments, neither of which were significantly different from each other. CRM extractable copper in the copper acetate amended treatments continued to be significantly greater than in the unamended treatments. The AGS continued its increase in CRM extractable copper content, raising it from 19.93 µg cm^{-1} at t = 105 days to 24.67 µg cm^{-1} by the end. The other two amended blends saw decreases in extractable copper during the final phase, their end products containing 2.791 µg cm^{-1} and 0.900 µg cm^{-1} for the APS and ACS respectively.

Comparison of CRM Extractable Copper with Sequentially Extracted Copper

Copper extracted from all blends, treatments and sampling times using the chelating resin membrane burial approach was correlated with copper extracted from the same materials using the Tessier *et. al.* (1979) sequential extraction

scheme. Results of the correlation analysis are given in Table 3.26 and Figure 3.13.

All correlations were found to be very highly significant ($p<0.001$), demonstrating that, on average, there is a straight line relationship between the $\log_{10}$ values of both types of measurement for all individual fractions, for the sum of fractions 1 and 2, as well as the sum of all 1-5 fractions.

Table 3.26 Correlation Coefficient (r) for the Relationship Between Log_{10} Cu Adsorbed by Chelating Resin Membrane (CRM) Strips and Log_{10} Amounts of Cu Extracted in Each Fraction of the Tessier *et. al.* (1979) Sequential Extraction Scheme.

	Log_{10} Amounts of Copper Extracted by Tessier Sequential Extraction Fractions						
	Fraction 1 Magnesium Chloride Extractable	Fraction 2 Sodium Acetate Extractable	Fraction 3 Hydroxylammonium Hydrochloride Extractable	Fraction 4 'Oxidisable'	Fraction 5 'Residual'	Sum of Fractions 1+2	Total Extracted (Sum of Fractions 1-5)
Log_{10} CRM Copper	0.874*	0.538*	0.596*	0.491*	0.537*	0.872*	0.708*

* Significant at the p = 0.001 level.

Scatter within the data, particularly for Fraction 4, produced fairly low correlation coefficient values. The fact that the values for p demonstrate very high significance is due entirely to the large sample size involved; the 69 observations used yielding a total number of degrees of freedom of 68. It is therefore likely that with a much smaller sample size significant correlations would not have been achieved.

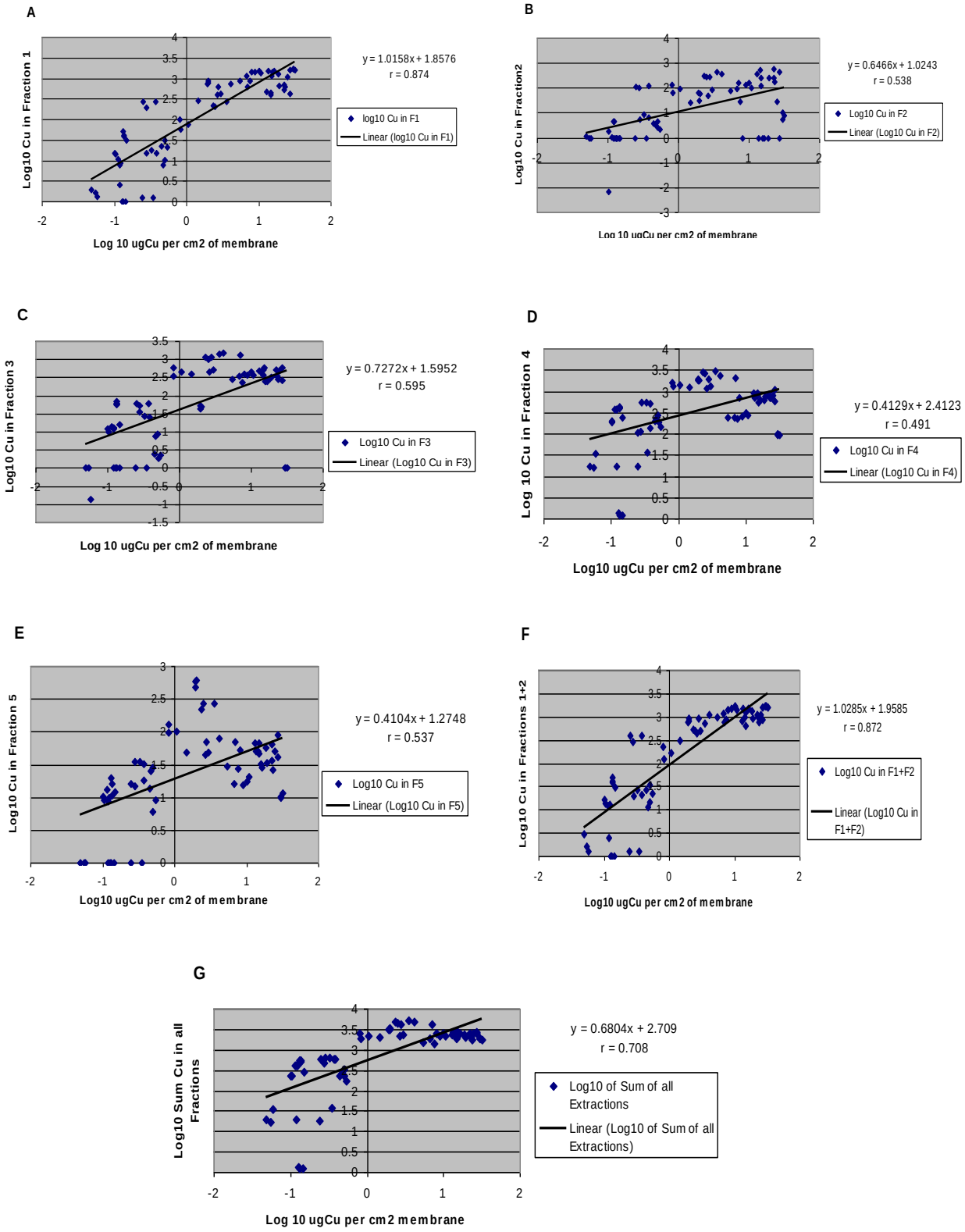


Fig. 3.13 Correlation Analysis of Log_{10} Cu Adsorbed by Chelating Resin Membrane (CRM) Strips and Log_{10} Amounts of Cu Extracted in Each Fraction. **A** = Fraction 1 ($MgCl_2$ Extracts), **B** = Fraction 2 (Sodium Acetate Extractable), **C** = Hydroxylamine Hydrochloride Extractable, **D** = 'Oxidisable' Fraction, **E** = 'Residual' Fraction, **F** = Sum of Fractions 1 + 2 and **G** = Sum of Fractions 1-5 of the Tessier *et. al.* (1979) Sequential Extraction Scheme.

3.3.3 Discussion

Use of chelating resin membranes to measure copper contained within these composting materials was a unique approach in that this technique had previously only been applied to soils and sediments. It is an approach based on mimicking the action of roots by measuring not just the ions in the immediate vicinity of the membrane but also the contribution of nutrient ion diffusion through the material tested to it (Skogley and Dobermann, 1996). It is also *in situ* and is therefore a non-destructive method. This is different to the approach of conventional extractions which are destructive and measure the nutrient pool as a whole (Liang and Schoenau, 1995).

DTPA-Anion Exchange Membrane, as used in this experiment, was determined as having a copper chelating capacity of just 2.96 μg cm^{-2}. However, it is clear from the results that the capacity of the membrane for copper binding, was not reached after insertion into these materials at FC. This must therefore lead to the conclusion that the original method for determining chelating ability was inappropriate. The copper solution used was copper chloride, a solution which effectively contains hydrated Cu^{2+} ions in solution. The copper solutions used to amend the composting materials were different. Copper-EDTA was found to have a particularly strong affinity for the membranes as evidenced by the unusually high quantities removed at t = 0 days, compared with the copper acetate and sulphate solutions. It was noted that membrane strips removed from these samples after 24 hours had acquired a blue mottled effect over

their surfaces. It is possible that the EDTA ligands present in the sample supplanted the DTPA ligands on the membrane and enhanced removal of copper from the material. EDTA-Anion Exchange Membrane has been seen to extract larger quantities of copper from soils relative to DTPA-Anion Exchange Membrane (Tejowulan *et. al.* 1994) and this was attributed to the greater ability of the ligand to remove organically complexed and oxide bound metals. What is perhaps more likely, particularly with the brown waste materials and even the grass/sawdust materials from t = 105 days onwards, is that the membranes adsorbed or absorbed large amounts of dissolved organic compounds, many of which held complexed copper ions. Brown and yellow-brown discolouration and mottling of the membrane surfaces was seen in a number of cases, particularly during the later sampling dates. Darkening of resin membranes has also been seen by others (Qian and Schoenau, 2002) and attributed to the accumulation of humic and fulvic acids within the membrane's lattice. Exchange sites are apparently left largely unaffected. However, if these trapped organic acids are still chelated with copper this may account for the increased quantities of copper removed from the membranes. However, only with further investigation of these phenomena would it be possible to draw firm conclusions.

Great lengths were taken to bring the materials obtained in this study to as near to field capacity as possible, as it is often reported that moisture content plays an important role in ion diffusion through soils and thus results obtained using resin membranes are affected. However, Western

Ag Innovations Inc who use the same ion exchange membrane in their PRS^{TM} probes go to no such effort. They make only a very subjective assessment as to whether their soil samples have reached FC before they insert their probes into the samples (Hangs *et. al.* 2002). Though their approach is a subjective one, they claim that nutrient supply rates are not significantly affected because supply rates to their PRS^{TM} measured during an early investigation were unaffected in the range of 70 to 120% FC for a range of soil textures (Schoenau *et. al*. 1993). Only at moisture contents below 70% of FC were the results adversely affected. For this reason, it is not felt that any variations away from the true FC's of the trial materials would have made significant differences to the results.

Precision of the CRM technique was variable, even compared with the Tessier scheme. Most values of coefficients of variation produced during the ANOVA analyses ranged from a 6.5% to 27.1% for the 4 grass/sawdust treatments, but were very poor for the unamended and copper acetate treated grass/sawdust, pig slurry/sawdust and sewage sludge cake/sawdust blends. These values ranged from 20.7% to an exceptionally poor 503.8% for results obtained at t = 105 days. Accuracy of resin testing results in soils has been commented on (Qian and Schoenau, 2002) and where resins are installed in unprocessed soils, a larger CV than normal is said to be expected as soil is not homogeneous. Western Ag Innovations Inc. (Western Ag Innovations, 2006b) claim that comparing the CV values from a 'homogeneous (i.e., dried, ground, and sieved) soil sample using a chemical extraction to CVs using PRS™-

probes is inappropriate.' They report the mean (n = 68) nutrient supply rate (µg 10cm^{-2} 24hrs) and CV values of a range of elements within a homogenised soil sample as determined in their laboratory using PRS^{TM} probes. CV values ranged from 9 to 27% (Copper was 19%) and they point out that *in situ* under field conditions CV's would be expected to be much larger owing to micro-scale variability, differences in temperature, pH and other environmental factors. They are also point out that the magnitude of the nutrient supply rate plays a very large part in determining the coefficient of variation – a variation of 2 µg/10cm^{-2} /24hrs for NO_3^- (mean of 12) will have a much larger CV than the same variation experienced with an element of a larger mean e.g. Ca^{2+} which had a mean of 1624. For this reason they suggest that it is more meaningful to discuss variation within individual sets of replicates. When this is approach is taken the CV values for each set of replicates (i.e. each block for data from t = 105 and t = 318 days) generated in this study are much closer to the 9-27% range (19% for copper) reported by Western Ag Innovations for their 'thoroughly homogenised' soil sample. All but four of the values (53 values) are below 28%, two values lie between 30 and 40% and the remainder (outliers) are between 56 and 62%. Though values are sometimes higher than those suggested, it must be remembered that the materials are different, that numbers of observations were much smaller (n = 3 for most sets of replicates) and the range of copper values much greater (0.03 – 32 µg cm^{-2}). Samples were reasonably homogeneous, but variability within the materials may still have been a problem, particularly with the sewage sludge

cake/sawdust blend. Although the cake had been sieved to 12.6mm and blended well with the sawdust, some of the particles of cake were still large compared with the dimensions of the membrane. Inhomogeneity may therefore have played a role in the larger CV values seen. Microbial activity within the materials is also a possibility given that the samples were analysed at field capacity and room temperature and may also have influenced the results. Certainly micro-organisms have been shown to compete with resin membranes for ions (Sulewski *et. al*. 2002).

During a 1-24h burial period (considered a 'short-term' burial – Western Ag Innovations Inc. 2006), the nutrient supplying power of the soil is mainly derived from the rapidly available labile nutrient pool. The membrane acts as a sink and provides a snap-shot of the readily available pool of the nutrient in question (Sulewski *et. al.* 2002; Western Ag Innovations Inc, 2006). Beyond this time length, or whenever the exchange sites of the membrane become saturated, the membrane starts to act as a dynamic exchanger with ions adsorbing and desorbing according to the prevailing conditions. In these circumstances the membrane acts as a source of ions in equilibrium processes (Qian and Schoenau 2002). Removing and replacing membranes in the same position in the same sample on a regular basis can be useful for assessing nutrient supply rates in soils over a period of weeks and months but this is not the aim here. As short-term burials have been found to correlate well with concentrations of available nutrients obtained by conventional chemical extractants (Western Ag Innovations Inc. 2006);

and as Schoenau *et. al.* (1993) found that membrane uptake of N, P, K and S after 24 hours burial exceeded the uptake found after a 1 hour burial; and because Western Ag Innovations Inc. employ a 24 hour burial period for their in-lab soil assessments (Hangs *et. al.* 2002), it was decided that a 24 hour burial period would be used in this study, rather than just the 1-hour used in other situations (e.g. Liang and Schoenau, 1994).

Given the fundamental differences between the chelating resin membrane and conventional single or sequential extraction approaches, it would be expected that the results obtained from the use of chelating resin membranes (CRM's) in this experiment might be very different to those results obtained from the application of the Tessier scheme. In fact, it is an opinion widely held among resin membrane use researchers that results for CRM adsorbable copper cannot be directly compared with those from conventional extractions (Drohan *et. al.* 2005) because the amounts determined are expressed per unit surface area of membrane strip rather than per unit weight (Tejowulan, *et. al.* 1994) of material and are really a measure of ion flux or supply to the resin (Greer *et. al.,* 2003). Any differences or similarities seen can therefore only be considered as relative. Tejowulan *et. al.* (1994) did, however, correlate copper extracted from Saskatchewan and Indonesian soils using the same BDH anion exchange resin membrane in both DTPA and EDTA chelating forms with copper extracted using conventional chemical extractions. Copper extracted with resin membrane in the DTPA form was found to be significantly linearly related to copper extracted from Saskatchewan soil

using DTPA-TEA (r = 0.76***) and EDTA-$(NH_4)CO_3$ extractions (r = 0.70***) and for copper extracted from an Indonesian soil by DTPA-TEA (r = 0.799***) and EDTA-$(NH_4)CO_3$ (r = 0.72**). In this compost trial, positive, highly significant correlations were obtained between CRM extractable copper and magnesium chloride extractable copper (Fraction 1 of the Tessier scheme) and CRM extractable copper and the sum of the magnesium chloride and sodium acetate extractable copper (Fractions 1 and 2 of the Tessier scheme). It is with these correlations in mind that the results of the CRM analysis are discussed.

Group A: Grass/Sawdust Blend - Unamended, Copper Acetate, Copper Sulphate and Copper EDTA Treatments

At t = 0 days, very small quantities of copper were adsorbed onto the CRM's (0.135 µg cm^{-2}). This increased during the first phase of composting to 0.237 µg cm^{-2} but then appeared to decrease again during the second phase to even smaller adsorbable quantities than at the start. In contrast, the Tessier scheme failed to detect any copper in t = 0 days materials other than in the oxidisable fraction. Over the course of composting, magnesium chloride extractable copper increased from 0.0 mg kg^{-1} at t = 0 days to 1.68 mg kg^{-1} at t = 105 days but, unlike the CRM adsorbable copper, it then remained effectively unchanged during the second phase. Copper in green waste materials has been found to be predominantly associated with organic forms (Greenway and Song, 2002b), but with some of it also found in easily extractable forms which

decrease over time. The CRM method has therefore detected the easily extractable forms present at the start of composting and also detected the increase in easily extractable copper which occurred during the first phase of composting. In this respect, the CRM method appears to be a more sensitive technique than the Tessier scheme. It does not, however, account for the decrease in adsorbable copper seen during the second phase. Overall, though, these changes were so tiny that it is possible they may not even have been significant, and as there are no other reports in the scientific literature to compare the results with, any conclusions drawn must be tentative ones.

Changes in CRM adsorbable copper within the AGS were very different, both in terms of the amounts removed and the extent to which they changed during the experiment. The CRM's removed significantly more copper from all the copper amended treatments than from the unamended control, and all were significantly different from each other. Adsorbable CRM copper was in the order EGS > SGS > AGS > UGS with the EGS containing more than 3 times the amount of CRM removable copper than the SGS and AGS. Possible reasons for this large difference between the EGS and the AGS and SGS samples have already been discussed.

Over the first phase, the EGS substantially decreased in CRM adsorbable copper, while the other two copper amended treatments increased their CRM adsorbable copper contents. During the second phase the AGS and

EGS treatments increased a little, while the SGS effectively reached a plateau. Such increases in CRM available copper in the AGS and SGS treatments during the first phase of composting were not reflected in corresponding increases in magnesium chloride extractable copper in the Tessier scheme, nor in the sum of copper in fractions 1 and 2 of the Tessier scheme. Copper in these treatments, as extracted during Fraction 1 of the Tessier scheme, decreased during composting, with the greatest changes occurring over the second phase of the experiment. Only the results for CRM available copper in the EGS treatment bore any kind of similarity to copper extracted in Fraction 1 (as well as the sum of fractions 1 and 2) of the Tessier scheme in which consistent decreases in magnesium chloride extractable copper were seen throughout the whole composting period. CRM extractable copper was also found to decrease over time, but only during the first phase.

The AGS and SGS treatments behaved in a similar manner, particularly during the first phase, and the EGS behaved rather differently, again, particularly during the first phase. These differences are as a result of the differing natures of the copper treatments (as explained in section 3.2.3.3). To this extent, the CRM copper results mimic those of the magnesium chloride fraction of the Tessier scheme, although it must be borne in mind that in the Tessier scheme, the trend for all the copper amended treatments was for the copper to redistribute to less easily available fractions.

Group B: Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends – Unamended and Copper Acetate Amended Treatments

Copper in the unamended and copper acetate treated grass/sawdust material has already been discussed in detail in the last section. It is therefore only considered here for comparison with the brown waste blends (pig slurry/sawdust and sewage sludge cake/sawdust blends).

At t = 0 days, the UGS appeared to contain larger quantities of magnesium chloride extractable copper than the APS (although no significant difference was established), which, in turn contained more than the ACS. In a similar manner, CRM adsorbable copper was of the order AGS (6.57 μg cm^{-2}) > APS (1.975 μg cm^{-2}) > ACS (0.301 μg cm^{-2}), and all were significantly different from each other. The smaller quantities of adsorbable and magnesium chloride extractable copper in the brown waste blends are likely to be due to the immediate bonding of the added copper with organics already present in the materials.

Changes in CRM adsorbable copper in the UPS and UCS did not follow the same patterns as those of the magnesium chloride extractable fractions of the Tessier scheme. The UPS gradually increased in CRM adsorbable copper over the whole composting period, whereas, magnesium chloride extractable copper sharply increased during the first phase of composting and then sharply decreased during the second

phase. The sum of fractions 1 and 2 of the Tessier scheme also show the same pattern. In the UCS, CRM adsorbable copper decreases during the first phase and then increased by almost the same amount during the second. Extractable copper in fraction 1 (and the sum of fractions 1 and 2) of the Tessier scheme declines during the first composting phase but then decreased further, and to a much greater extent, during the second phase.

The trends in CRM adsorbable copper within the copper acetate amended brown waste blends more closely resemble the results obtained by the Tessier sequential extraction scheme. Membrane adsorbable copper in the APS sharply increases by a factor of 2.35 during the first phase of composting and then decreases over the second phase by a factor of 1.66. This trend is not seen in the magnesium chloride extractable fraction of the Tessier scheme (copper within fraction 1 decreases throughout the process). However, when copper in the first two fractions of the scheme are added together, a trend similar to that observed with the CRM adsorbable copper is seen. Extractable copper in these two fractions increases from 841 μg cm^{-2} at t = 0 days to 938 μg cm^{-2} at t = 105 days (an increase of a factor of 1.12) and then decreases to 579 μg cm^{-2} by t = 318 days (a decrease of a factor of 1.62 – very similar to that seen in CRM adsorbable copper). The ACS treatment behaved similarly. CRM adsorbable copper increased by a factor of 7.84 during the first phase and then decreased by a factor of 2.62 during the second. Magnesium chloride extractable copper alone also increased

during the first phase and decreased during the second. However, the sum of fractions 1 and 2 more closely resembled the changes seen in CRM extractable copper. Copper in fractions 1 and 2 increased, though by a much smaller degree (a factor of 1.17) during the first phase and then decreased during the second phase by a factor of 2.40 (similar to that seen in the CRM adsorbable copper).

It is therefore possible to conclude that the use of chelating resin membrane under these conditions, in the analysis of these particular copper acetate treated brown waste blends, yields results which are comparable with copper extracted from fractions 1 and 2 of the Tessier sequential extraction scheme. For untreated brown waste blends and also for all treatments of the green waste materials, changes over time in CRM adsorbable copper do not correspond well with changes either in fraction 1 or in the sum of copper in fractions 1 and 2 of the Tessier scheme.

No other data for use of CRM's in determining heavy metals in compost is available for comparison and so the results are difficult to interpret. Lack of comparative information on the use of resin membranes in soil research is also a problem and has been commented on by others (Drohan *et. al.* 2005). Further work in this area is therefore necessary. Particularly important is the determination of changes in the adsorptive capacity of the chelating resin membrane under different conditions and whether or not the presence/absence of humic substances plays a

significant role. Investigating whether or not variability could be reduced by using larger pieces of membrane where samples lack homogeneity would also be very useful. More interesting though would be to investigate changes in the supply rate of copper over time during the composting process in much greater detail. Successive pieces of membrane, sampled at more regular intervals within a large compost windrow, placed in exactly the same position so as to determine the cumulative supply of copper during the process would yield more information. This would be difficult to achieve given the friable nature of the material. Membrane removal and replacement would be difficult and there would be the high probability of a lack of uniform contact between the membrane and the composting materials to be overcome (probably by having many replicate membranes in place). However, if achieved, such an investigation would yield more information on the cumulative supply of copper (or other nutrients) throughout the composting process and could easily be correlated with plant uptake.

On reflection, use of these membranes in routine testing of composts is not likely to be very practical, even if the results obtained do give a clearer picture of ion supply to plant roots. Assessing the water required to bring samples up to field capacity is very time consuming and onerous, whilst simply air drying samples and then performing a simple chemical extraction is not. Western Ag Innovations Inc. were, in 2002, developing a ‘wetting table’ on which to bring many hundreds of soil samples up to field capacity in a far more objective manner (Hangs *et. al.* 2002). Any

commercial laboratory seeking to employ the use of resin membranes in their routine procedures ought really to use this kind of apparatus, rather than take either the previously used subjective approach adopted by Western Ag Innovations, or the more accurate but more time-consuming approach used in this experiment. Determining FC is certainly the rate limiting step. If this can be overcome then the procedure is very straightforward and would be of practical use where sufficient material is available for testing.

Summary of Findings

- Determination of the total chelating capacity of the prepared CRM's by saturating with an aqueous solution of copper chloride did not adequately describe their total chelating capacities when exposed to composting materials.
- Variability within the data set should be considered within groups of replicates, rather than in terms of the whole data set.
- For all observations within the data set, CRM adsorbable copper correlated strongly and highly significantly with copper extracted from fraction 1 of the Tessier sequential extraction scheme as well as copper in the sum of fractions 1 and 2 of the Tessier scheme.
- Copper acetate amended brown waste treatments compared well with copper extracted from the sum of fractions 1 and 2 of the Tessier sequential extraction scheme.

- For untreated brown waste blends and also for all treatments of the green waste materials, changes over time in CRM adsorbable copper do not correspond well with changes either in fraction 1 or in the sum of copper in fractions 1 and 2 of the Tessier scheme.
- Limitations of the procedure included the time taken to determine the field capacity as well as the possible lack of homogeneity within the samples compared with the size of the membrane strips.
- If the 2 principal limitations of the procedure could be overcome, then the use of exchange resin membranes for the routine testing of composts and other media within the laboratory could be a viable option.

3.4 Total Cu, C, S and N

3.4.1 Materials and Methods

Total copper in all the samples was measured by x-ray fluorescence spectrometry (XRF) and not by acid digestion as it was felt to be too time-consuming an approach. Oven dried samples were ground and then pulverized with stainless steel ball bearings within a 25ml polyethylene container (Kartell) in a ball mill (Griffin, UK). Pellets were prepared by mixing 1g of pulverised sample with 4g of cellulose (Sigma product C6288) and then pressing the mixture into 40mm discs using a Graseby Specac Press set at 12 tonnes of pressure. Copper in the preprared samples was determined by XRF in a helium atmosphere on a Spectro XEPOS XRF machine with palladium anode and set to 5 minutes per scan, 3 scans per sample. The manufacturer's detection limit for copper was reported as $1mgkg^{-1}$. Calibration of the instrument was achieved using a range of 74 environmental standards and X-Lab Pro version 2.4 was the associated software.

Carbon and sulphur were determined on whole samples by total combustion in a LECO SC-144DR Total Carbon and Sulphur analyser. Total nitrogen was also determined in a similar manner by the Dumas method using a LECO FP-528 Total Nitrogen analyser. C/N ratios for each sample were calculated from the total carbon and nitrogen results.

Spearman's rank correlation (adjusted for tied values) was performed to see if the various ways in which copper content and distribution had been determined could be associated with total carbon, nitrogen, C/N ratio, sulphur and other parameters, including measurements made on water extracts of the materials. Details of the water extraction analyses are addressed in Section 3.6 and full correlation matrices with their correlation coefficient values and their significances are given in Tables 1 and 2 of Appendix X. The software program Genstat (9th Edition) was used for the calculations.

3.4.2 Results

Results for the total carbon, nitrogen, sulphur and C/N ratios are presented in Figures 3.14 and 3.15. Those for copper by XRF are in Fig 3.16 where they are compared with the total amount extracted by the Tessier sequential extraction scheme.

Total Carbon

For the grass/sawdust materials, carbon content in all treatments appeared to increase during composting. However, changes were small and so did not appear from the 95% confidence intervals to be significant.

Carbon content for the pig slurry/sawdust materials decreased during the first phase of composting. Increases were seen during the second phase, although for both 105 and 318 days there did not appear to be any differences between the two treatments.

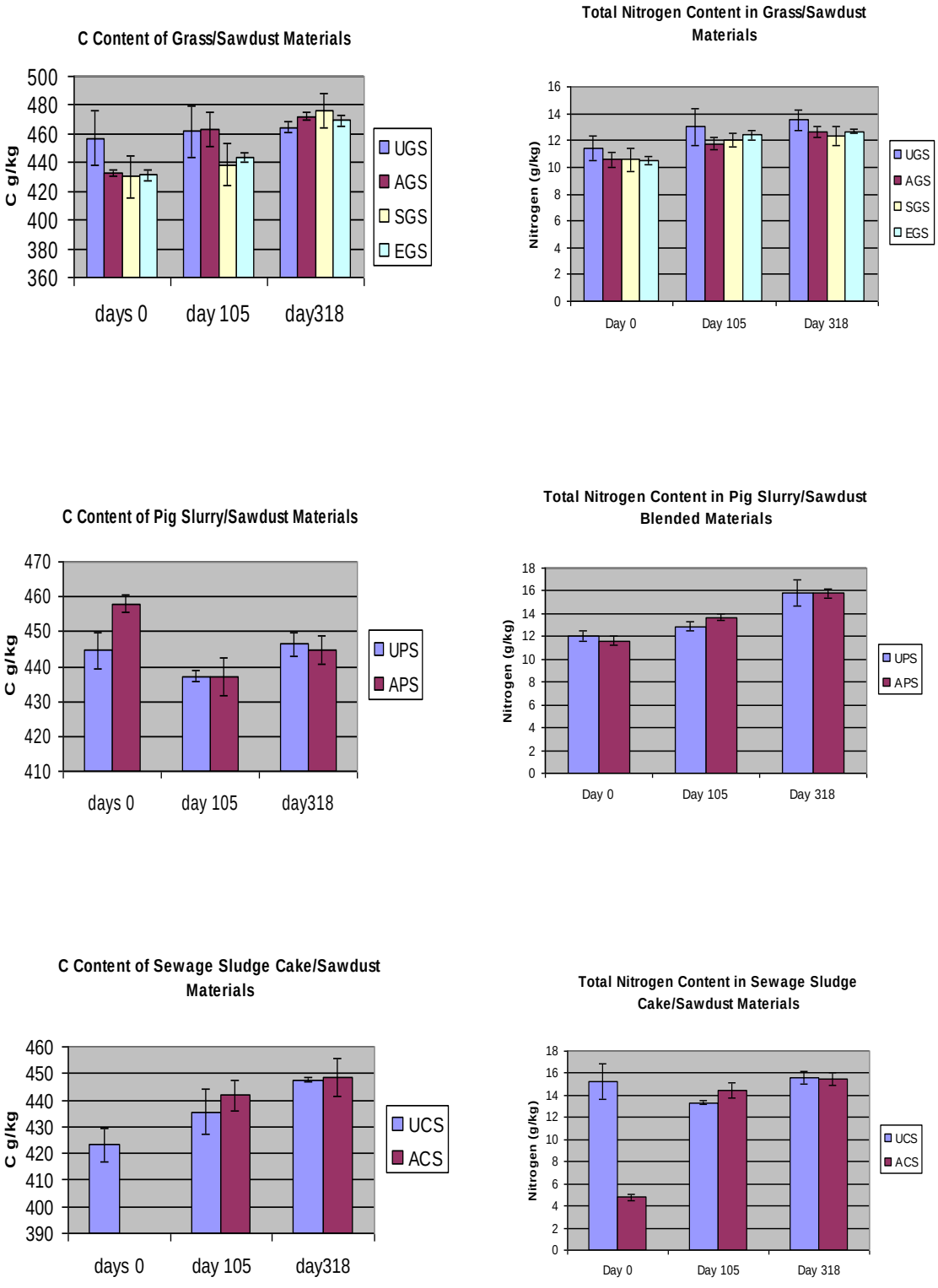
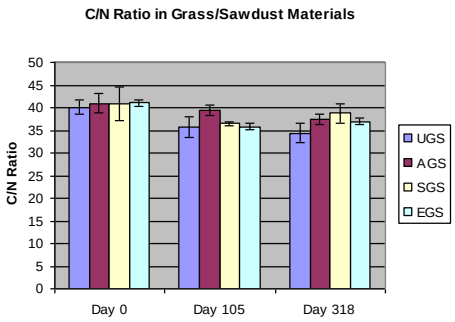
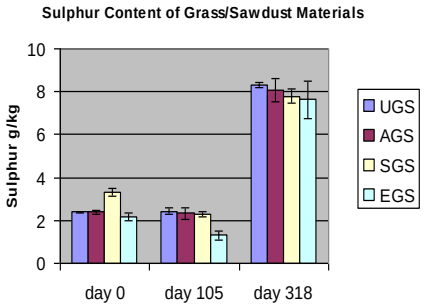
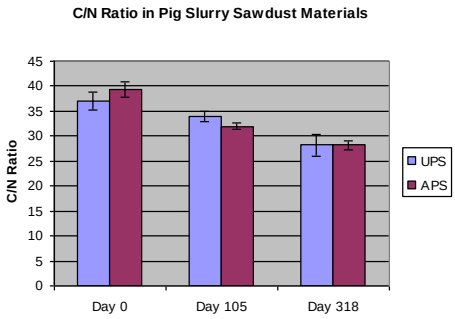
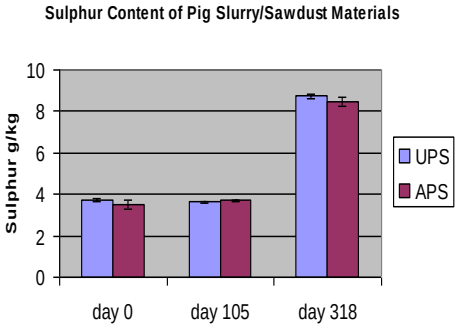


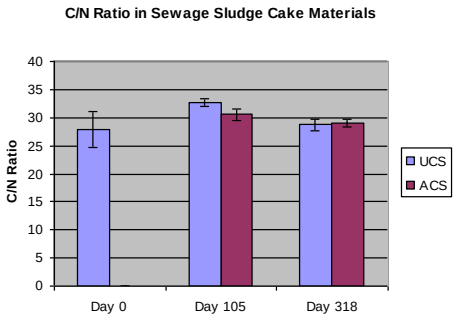
Fig. 3.14 Total Carbon and Nitrogen Contents of All Blends and Treatments Sampled at 0, 105 and 318 Days. Error bars are ±95% Confidence Intervals.











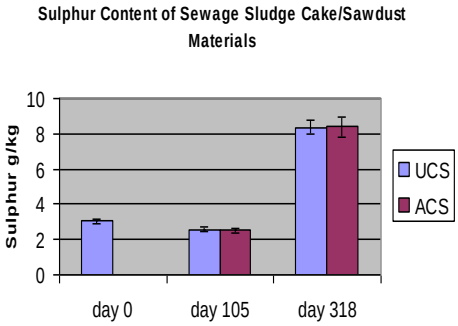


Fig. 3.15 Carbon/Nitrogen Ratios and Total Sulphur Contents of All Blends and Treatments Sampled at 0, 105 and 318 Days. Error bars are ±95% Confidence Intervals.

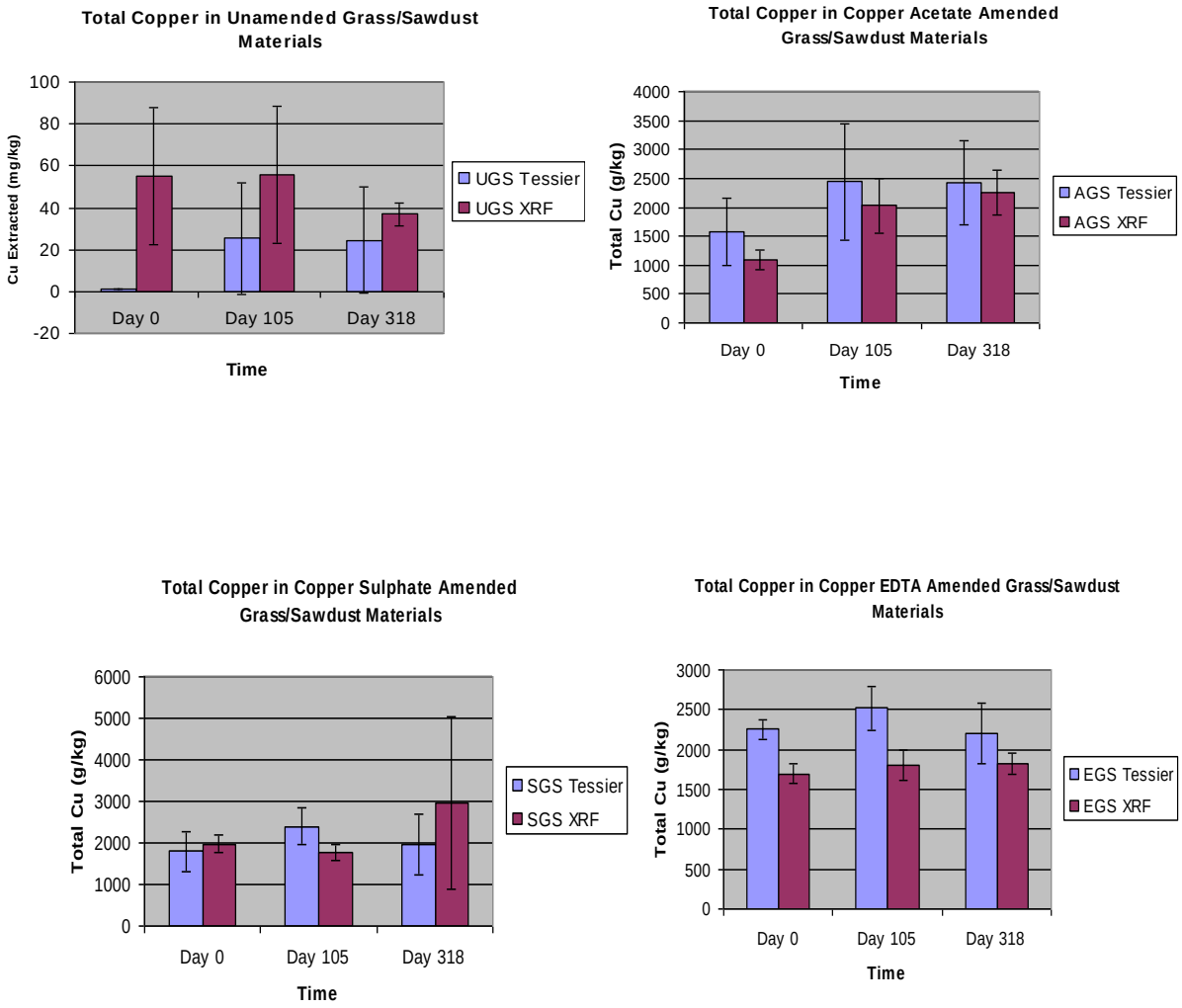


Fig. 3.16 Total Copper Content as Determined by XRF of all Treatments of Grass/Sawdust Material, Sampled at t = 0, 105 and 318 Days, Compared with Copper Sequentially Extracted using the Tessier scheme. Error bars are ±95% Confidence Intervals.

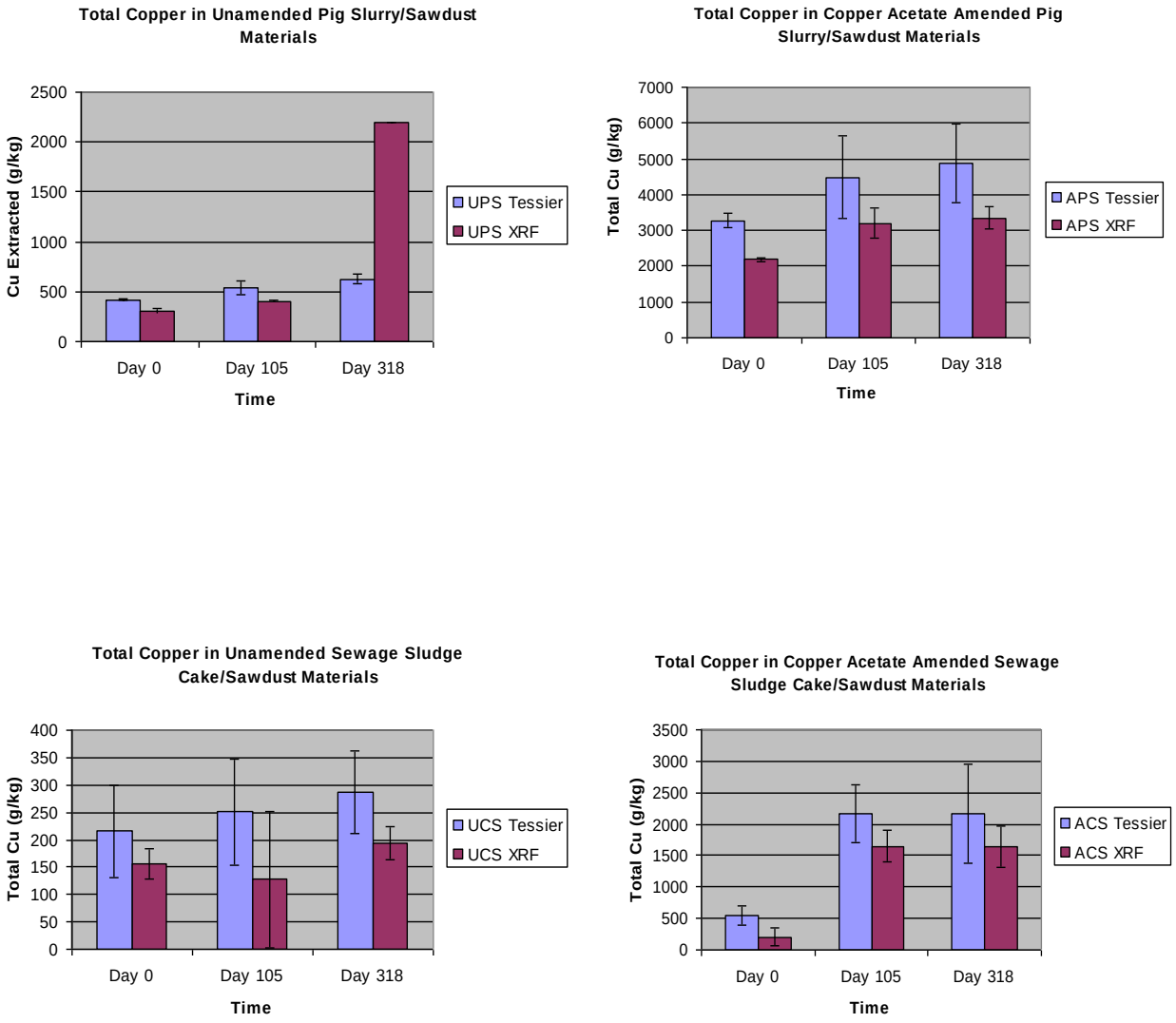


Fig. 3.17 Total Copper Content as Determined by XRF of Unamended and Copper Acetate Treated Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Materials, Sampled at t = 0, 105 and 318 Days, Compared with Copper Sequentially Extracted Using the Tessier Scheme. Error bars are ±95% Confidence Intervals.

Carbon within the sewage sludge cake/sawdust materials increased during composting. A clear increase in carbon content between 0 and 318 days could be seen for the UCS. Unfortunately, owing to lack of sample material, no data could be obtained for the ACS blend at t=0 days. However, it seems likely that the ACS would have displayed similar behaviour as the UCS, especially as there were no clear differences between the two treatments at both 105 and 318 days.

Total Nitrogen

No differences were seen between any of the treatments of the grass/sawdust materials at any of the sampling times. The trend over time was one of an increase in total nitrogen. However, changes were very small and so any differences seen may not have been significant, particularly for the unamended materials. Increases seemed more apparent in the copper treated materials between 0 and 105 days, but not between 105 and 318 days.

Nitrogen contents in the pig slurry/sawdust materials increased during composting. No differences were observed between the two treatments at 0 and 318 days, although at 105 days the APS appeared to contain slightly more than the unamended material.

Clear increases were seen in the ACS during the first 105 days of the experiment. A smaller increase was seen during the second phase but

this did not appear to be significant. The UCS behaved differently and seemed to fluctuate, first decreasing in total nitrogen and then increasing.

C/N Ratios

C/N ratios were seen to decrease in all materials during the composting process. Only in the SGS and EGS materials did this seem to be less clear although this may have been due to variation within the data. Overall, changes were small for all materials and may have been due to the C/N ratio being higher at t=0 days than would normally be recommended.

Total Sulphur

Large increases in total sulphur were seen in all samples during the second phase of composting. No differences were seen between any of the treatments in the brown waste materials at the different sampling times. Differences were more apparent however in the different copper treatments of the grass/sawdust materials. Decreases in sulphur were seen in the SGS and EGS during the first phase of the experiment, whilst the UGS and AGS did not appear to change during this period. By t = 318 days all of the copper treatments for this blend contained very similar quantities of total sulphur.

Total Copper by XRF

XRF spectrometry determination of copper within the UGS materials gave larger results than the sum of the five fractions of the Tessier sequential extraction scheme. However, variation among the replicates was high and so differences were not measurable at 105 and 318 days. XRF results for samples taken at 0 days were much greater than the amount extracted by the Tessier scheme. This was either due to incomplete extraction of the starting materials or insufficient sensitivity of the XRF machine at this level of copper. Copper was seen to increase in the AGS during composting. However, variation within the data meant that no significant differences were seen between the two methods for any of the three sampling dates. No differences were seen between the two methods at 0 and 318 days in the SGS, though at 105 days, the XRF determined copper was lower than the extractable amount. The same pattern for the XRF determined copper in the SGS was also seen in the EGS. Whereas, copper extracted at 105 days by the Tessier scheme was greater than at day 0.

Total copper determined in the UPS appeared to increase during composting, regardless of the method used. XRF values were lower than the total extractable amount at 0 and 105 days, but exceeded them at 318 days. The total extractable amount in the APS was greater than the amount determined by XRF at 0 and 318 days. It might also have been the case at 105 days. However, variation within the data for extracted copper meant that a significant difference could not be established.

Increases in total extractable copper were seen during the composting of the UCS; although variation within the data suggests that these differences are not significant. Total copper by XRF gave lower figures than those from the five extractions of the Tessier scheme. However, variation within the data prevented any kind of trend from being suggested. In the copper acetate treated materials, total copper increased over time. XRF values were again lower than the total extractable amounts but these differences were only clear at 0 days.

Regression analysis (Fig. 3.17) of the results from the two methods gave a value for r^2 of 0.8168 and a value of $p \leq 0.001$. From the regression equation, c = 222.4, indicating that, averaged over all the samples,

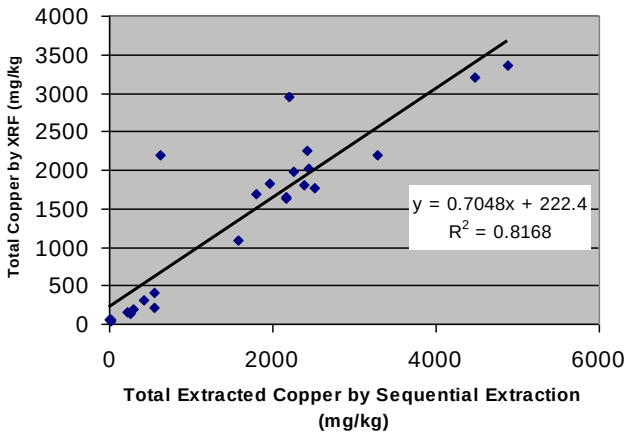


Fig 3.18 Regression of Total Extractable Copper by the Tessier Sequential Extraction Scheme and Copper by XRF

copper determined by XRF gave higher values of copper than the sequential extraction method. Therefore, despite differences between some of the samples, there is overall a strong relationship between the two methods.

3.4.3 Discussion

C/N ratios of composts are used as indicators of maturity because as composting proceeds, oxidation and condensation of organic matter leads to losses of C and so C/N ratios tend to decrease (He *et. al.* 1995). Decreases in C/N ratios have been reported during the composting of swine manure (Hsu and Lo, 2001), municipal solid waste (Chefetz *et. al.* 1998); wood shavings, sawdust and peat moss (N'Dayegamiye and Isfan 1991) and sewage sludge (Amir *et. al.* 2005). Overall losses of total organic carbon in this experiment did not occur. Total nitrogen was seen to increase over and above the rates of carbon increases and so small reductions in C/N ratios were seen for these materials. In mature composts C/N ratios of no more than 20:1 are usually acceptable (Mathur *et. al.* 1993; WRAP, 2005b), the aim being to acquire an end product which, when applied to the soil, releases mineral nitrogen rather than immobilising soil nitrogen (Mathur *et. al.* 1993). The end products in this experiment all exceed this value and so would normally be considered immature. Soluble carbon was seen to decrease (see section 3.6). The small observed increases in total organic carbon are therefore relative and suggest that the bulk of it resided within the sawdust. Sawdust was a

key substrate in all the blends, and seemed resistant to decomposition, despite the additional presence of nitrogen containing substrates (grass, pig slurry and sewage sludge cake). Co-composting of lignaceous materials (N'Dayegamiye and Isfan 1991) with cattle manure resulted in decreases of C/N ratios and was also very slow. Even after 36 months of composting the C/N ratio of a sawdust/manure mix was still around 35:1, having decreased from just over 50:1. Despite the high C/N ratios and other indicators of immaturity of their end products, the composting process significantly reduced phytotoxic effects, enabling corn and faba beans to be grown.

Increases over time in the copper content of the samples were observed, thus supporting widely reported occurrences within the literature of the concentrating effects of composting processes upon heavy metal contents. Concentrations in the composting of swine manure (Hsu and Lo, 2001) increased from 343 to 976 mg kg^{-1}; in cattle manure (Inbar *et. al.* 1993) from 69 to 79 mg kg^{-1} and in sewage sludges (Qiao and Ho 1997). Increases are due to losses in organic matter (Anid 1986) and have been significantly negatively correlated with its reduction (Szymanski, *et. al.* 2005). This was not the case in this experiment. Copper extractable from the green waste materials by fraction 1 of the Tessier scheme was significantly negatively correlated with total organic carbon, whilst XRF copper was positively correlated with organic matter content for the same samples (section 3.6). No other significant

correlations between total copper and carbon or organic matter were found. This may be a result of the very slow decomposition rates of the materials. Left to mature for longer, a relationship between these parameters may have been seen.

Total sulphur was seen to increase during composting, particularly during the second phase of the experiment, alongside the decreases seen in the volatile solids contents. As with the carbon, this is possibly due to the bulk of it residing in the largely undecomposed sawdust. An initial measurement of the sawdust gave it a sulphur content of 7.63 g kg^{-1} (± 0.50 g kg^{-1}; ± 1 s.e.). Amounts of sulphur found at the end of the experiment were often close to this value. The EGS, for example, had a total sulphur content of 7.62 g kg^{-1} (± 0.90 g kg^{-1}; ± 95 C.I.) at t = 318 days. Values for total sulphur were positively correlated with XRF determined copper in all samples. It could be that the presence of copper is in some way inhibiting the composting process or, the copper became bound to sulphur containing functional groups within the humic fraction of the material. Sulphur is known to be an important, though often ignored, element within humic and fulvic acids (Stevenson, 1994). As copper is known to preferentially bind with these acids, this might also be the reason for the significant correlation.

3.4.4 Summary of Findings

- Total carbon, nitrogen and sulphur were all seen to increase during the composting process.
- C/N ratios decreased, probably as a result of only small comparative losses of soluble carbon and the concentrating effect of the process on the nitrogen content of the materials.
- C/N ratios of the end products exceeded 20:1, suggesting that the materials were still immature. This is likely to be due to the use of sawdust as a carbon source which decomposes very slowly.
- Total copper measured by XRF correlated with total sulphur in all the materials and with organic matter in the green waste materials. No other correlations were seen.

3.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

3.5.1 Materials and Methods

Oven dried samples were ground and then pulverized with stainless steel ball bearings within a 25ml polyethylene container (Kartell) in a ball mill (Griffin, UK). Sub-samples approximately 1g in weight were then ground in an agate mortar with KBr at a ratio of 1 part sample to 10 parts KBr. The resulting mixture was then pressed into discs using a Graseby-Specac press operated at 12 tonnes. All samples, KBr and prepared discs were kept in a drying oven to prevent uptake of moisture.

FTIR analysis of the prepared discs was carried out on a factory calibrated Nicolet Impact 404 FTIR Spectrophotometer and using Omnic software. % Transmission through the sample discs of all wavenumbers from 400 to 4000 cm^{-1} was recorded and results converted to absorption values using the equation:

$$A = \log_{10}(100/T)$$

where A equals the value for absorption and T the value for % transmission.

To effectively compare spectra, all results were normalised to wavenumber 3386.30 cm^{-1} (this being assigned an absorption value of

1.0), this having the largest absorption for almost all samples. Relative comparisons between spectra could then be readily made.

Statistical analyses were performed on a selection of key wavenumbers. Details of these wavenumbers and the functional groups to which they are often attributed are given in Table 3.27. Analysis of Variance (ANOVA), using Tukey's method of multiple comparisons between means at the 95% confidence interval was performed to compare differences between treatments. Results can be found in Appendix VI. Spearman's Rank Correlation (results in Appendix VIII) was also carried out to see if there was any relationship between absorption values of the same key wavenumbers and the amount of copper extracted from the samples. The software program Genstat® (9th Edition) was used for all statistical analyses.

Table 3.27 Selected Key Wavenumbers and Their Functional Groups (Paré *et. al.* 1999 and Provenzano et al 1998)

Wavenumber (cm^{-1})	Functional Groups
3400	H-bonded OH, possibly water
2920	aliphatic CH3 stretch (lipids)
2850	aliphatic C-H stretch (lipids)
1643	aromatic strong band of COO- (most likely to be proteins).
1431	aliphatic C-H groups from incorporated structures like fatty acids and waxes
1319	strong band of COO-
1240	aromatic shoulder, C-OH of COOH phenols
1099	C-O stretch of polysaccharide bands
1030	C-O stretch of polysaccharide bands

3.5.2 Results

Group A: Grass/Sawdust - Unamended, Copper Acetate, Copper Sulphate and Copper EDTA Treatments

At t = 0 days, both wavenumber and treatment effects were very highly significant ($p \leq 0.001$). Interactions however were not significant ($p=0.998$) and no significant differences were seen between treatments for individual wavenumbers. By t = 105 days, wavenumber effects were still very highly significant ($p \leq 0.001$) but treatment and wavenumber x treatment interactions were not significant ($p = 0.910$ and 1.000 respectively). Again, no significant differences were seen between treatments for any of the key wavenumbers. By the end of the experiment at t = 318 days, no significant differences were measured between any of the treatments for any of the wavenumbers. Wavenumber effects were very highly significant ($p \leq 0.001$), but treatment effects and interactions were not significant ($p= 0.536$ and 1.00).

Group B: Grass/Sawdust, Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Blends – Unamended and Copper Acetate Amended Treatments.

At t = 0 days, wavenumber effects were very highly significant ($p \leq 0.001$), but all other effects and interactions were not significant. No significant differences were seen between any of the absorbance values for any of the key wavenumbers.

Some differences became apparent by t = 105 days. Wavenumber and wavenumber x blend x treatment interactions were very highly significant ($p<0.001$) and significant respectively ($p= 0.015$). However, no significant differences were found between any of the treatments for any individual wavenumber.

By t=318 days wavenumber and treatment effects were very highly significant ($p\leq0.001$) but blend effects were not ($p=0.637$). Of the interactions, only blend x treatment interactions were significant ($p\leq0.001$). Once again, no significant differences were found between any of the treatments for any single wavenumber.

Changes Over Time – all Blends and Treatments

Changes over time for the whole spectra are shown in Figures 3.19 and 3.20. Spectra closely resemble those found by others (Provenzano *et. al.* 1998). Changes over time in the absorption values of the chosen wavenumbers in the various blends are given in Figures 3.21 and 3.22.

Most blends were seen to behave rather differently. In the green waste materials absorption values appeared to increase between t = 0 days and t = 105 days and then decrease again by t = 318 days. However, variation in the data meant that in most instances this could not be proven. Only at 1030 cm^{-1} was the decrease in absorption during the final

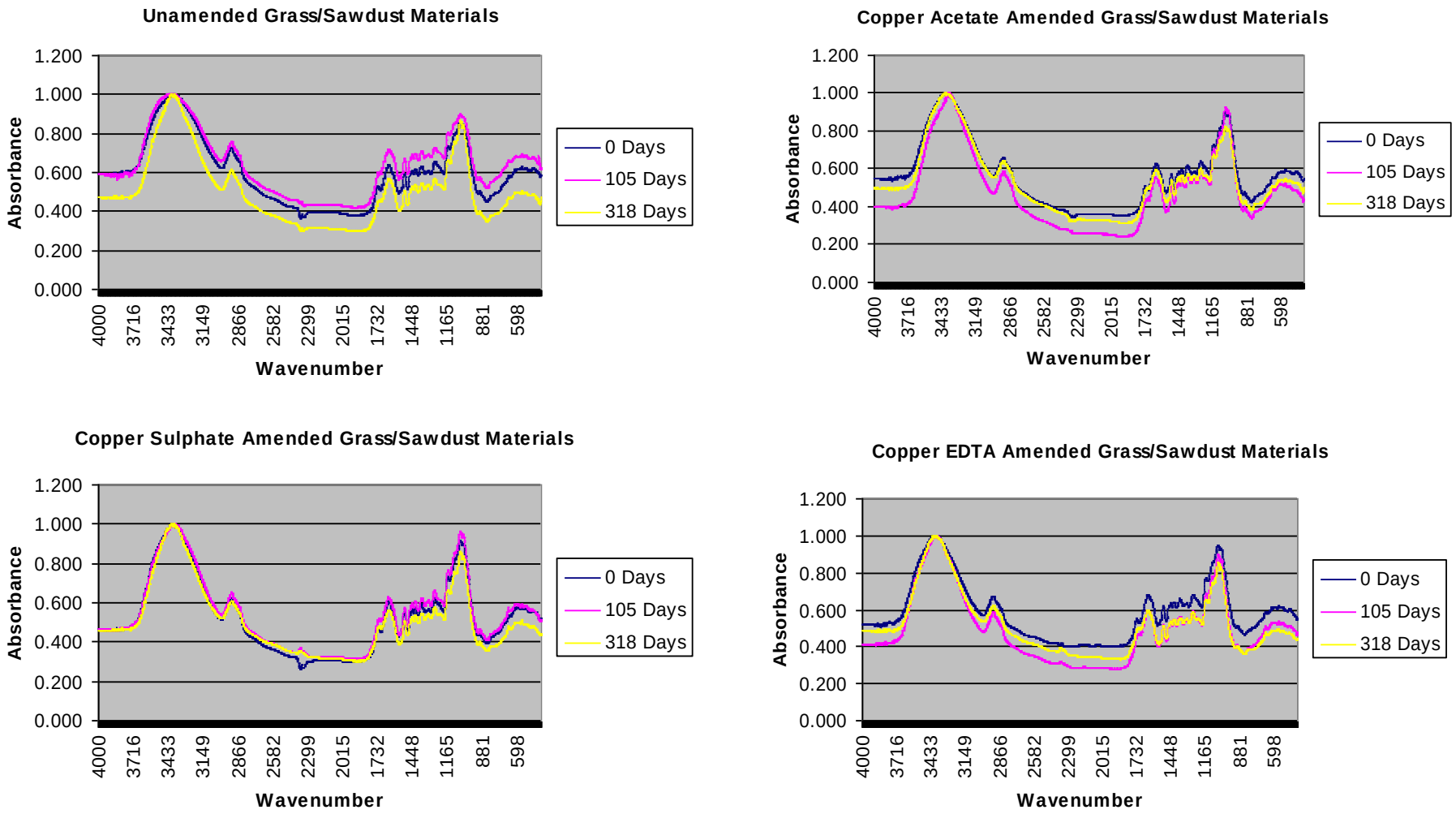


Fig. 3.19 FTIR Absorption Spectra for Grass/Sawdust Materials at Each Sampling Time.

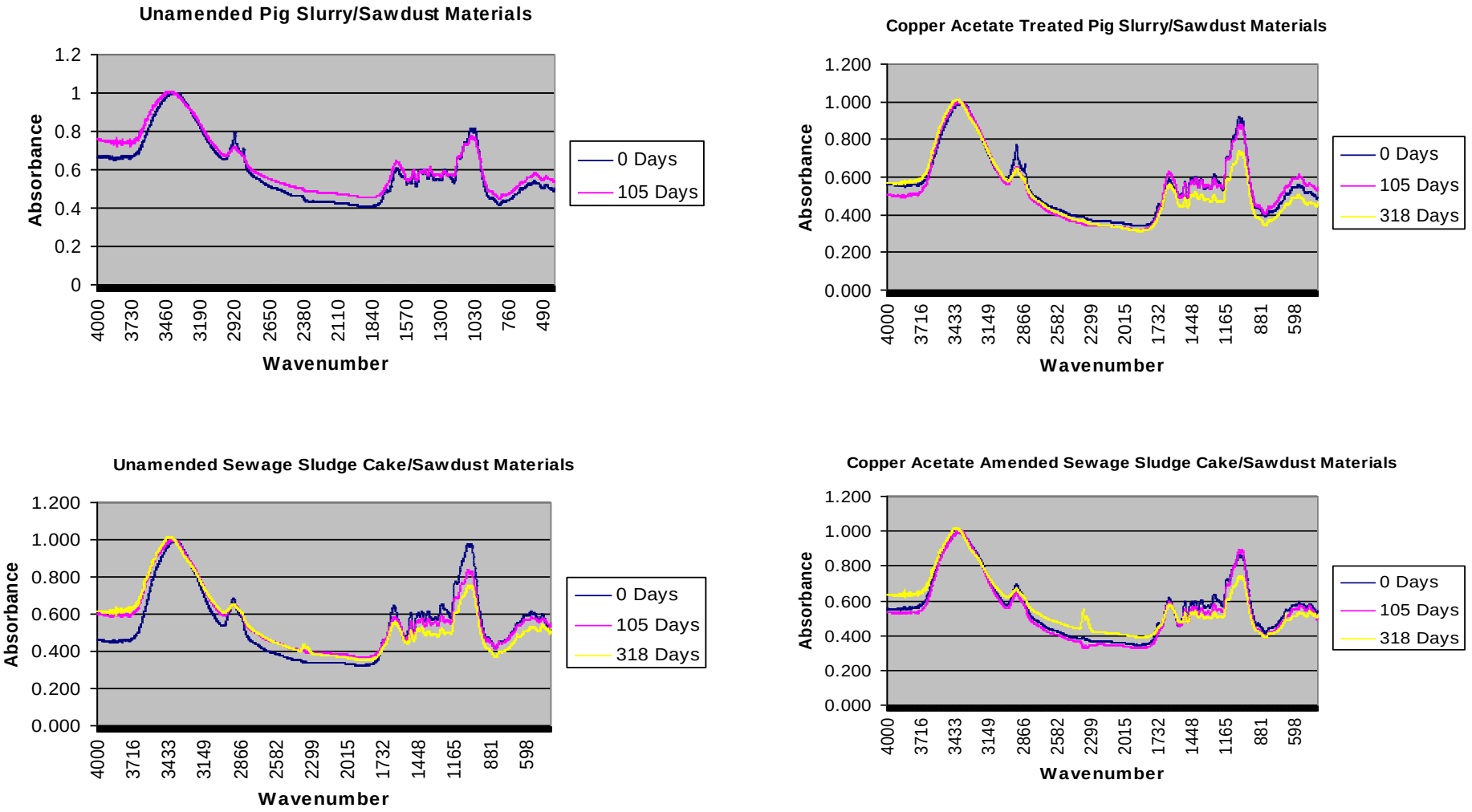


Fig. 3.20 FTIR Absorption Spectra for Unamended and Copper Acetate Treated Pig Slurry/Sawdust and Sewage Sludge Cake/Sawdust Materials.

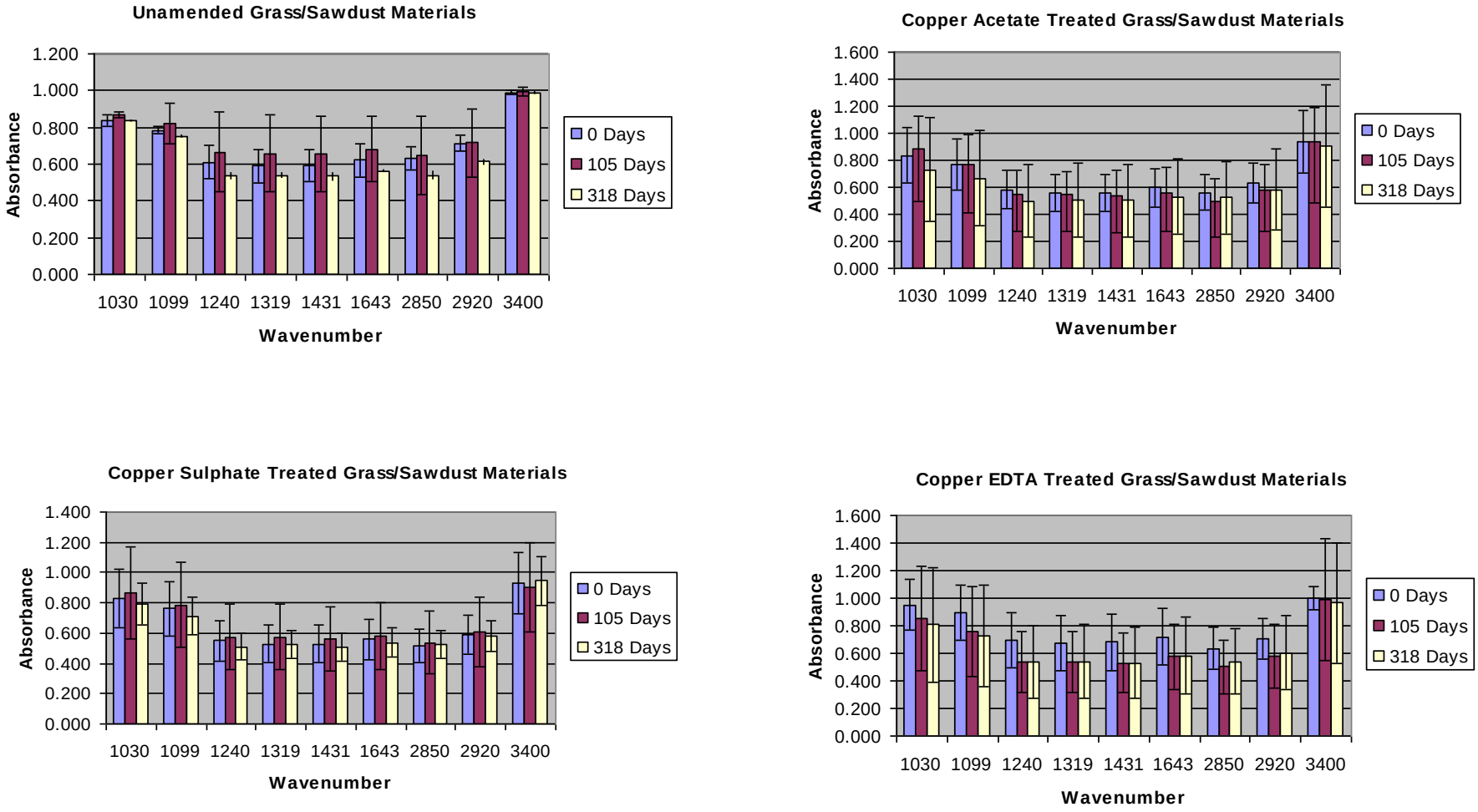


Fig. 3.21 Absorption at Selected Wavenumbers of Green Waste Materials by FTIR.

Error Bars = 95% Confidence Intervals.

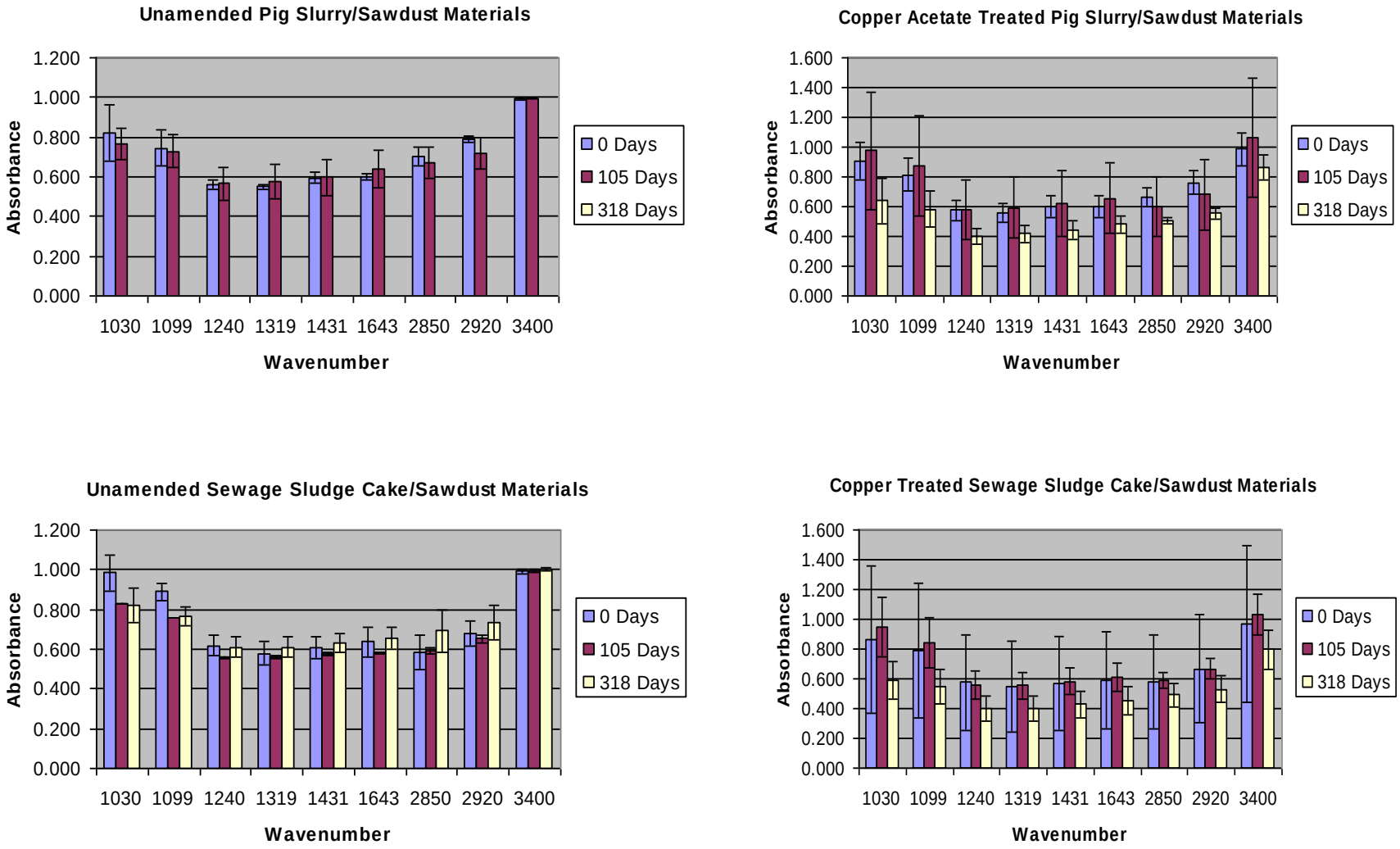


Fig. 3.22 Absorption at Selected Wavenumbers by Brown Waste Materials by FTIR.

phase significant at the 95% confidence interval.

With the AGS, SGS and EGS materials, variations in the data meant that no differences in absorption values were seen at any time point, for any wavenumber. Across the whole spectra, the UGS absorption values increased slightly during the first phase of composting and then decreased again to less than what they had been at the start. The SGS changed very little during composting, whereas the AGS and EGS materials appeared to exhibit large decreases in absorption during the first 105 days and then an increase during the final phase across the whole spectra. However, these changes are not likely to be significant given the variation observed in the data for the selected wavenumbers.

Similar observations were made of the brown waste blends. Absorption of the key wavenumbers did not change significantly over time for any of the treatments. Across the spectra, results suggested that changes had occurred. For example, results for the APS indicated substantial decreases in absorption in the 1020 – 1400cm^{-1}region by 318 days. But again, variation within the data for the selected key wavenumbers meant that differences between 105 and 318 days could not be reliably established.

Spearman's Rank Correlation Analysis – all Blends and Treatments

FTIR absorption values for the selected wavenumbers were correlated with copper extractable by 1) Fraction 1 of the Tessier sequential extraction scheme, 2) Sum of fractions 1-5 of the Tessier sequential extraction scheme, 3) Chelating Resin Membrane analysis and 4) Total copper by XRF.

Copper extracted by the four methods listed all correlated well with each other, values for p being either ≤0.001, or ≤ 0.050 or ≤0.010. Absorption values for the various wavenumbers also correlated well with each other, with p values being ≤0.001. Significant and highly significant negative correlations (p ≤ 0.050 and ≤0.010) were also achieved between total extractable copper by fractions 1-5 of the Tessier scheme and absorption values for all wavenumbers. Total copper as determined by XRF and absorption values were significantly (p ≤ 0.050) negatively correlated for absorption at all wavenumbers except 2850nm^{-1}

3.5.3 Discussion

By far the most dominant peak seen in all of the spectra was that in the 1000-1100 cm^{-1} region. This corresponds to C-O stretching of polysaccharides (Paré *et. al.* 1999) and, for these materials can be attributed to the presence of cellulose and lignin derived from the sawdust within the samples. Decreases in absorption bands in this area have been seen, particularly in the brown waste blended materials, suggesting decreases in the polysaccharide content of the samples. Similar

decreases in the polysaccharide content of composting materials have been seen (Provenzano et al ’98 – municipal solid waste composts; Inbar *et. al.* 1989 – cattle manure) and also attributed to decreases in cellulose rather than lignin (Durig *et. al.* 1988), owing to the fact that though lignin absorbs in this region, it does not do so with such intensity. Changes in this region are therefore more likely to reflect changes in cellulose content. Inbar *et. al.* (1989) also saw decreases in polysaccharide groups but with very little change in total lignin content. Carbohydrate degradation was the explanation given. Despite such decreases in polysaccharide content, all the materials remained predominantly aliphatic in nature, with carbohydrates being the major component. This has also been the experience of others (Inbar *et. al.,* 1989).

Fluctuations in absorption have been seen at 1643 cm^{-1} within all the green waste materials and the copper amended brown waste materials with an overall decrease being observed. However, variation within the data for these materials means that this trend is not proven. Bands around 1640 cm^{-1} correspond with aromatic C=C vibrations and C=O stretching of amide groups possibly originating from incorporated proteinaceous materials of which composts are rich (Provenzano et al ’98). Boyd *et. al.* (1979) after hydrolysing sludge fractions with 6N HCl found decreases in absorbance at 1645 and 1040 cm^{-1}, indicating the presence of proteins and carbohydrates. Decreases in absorption, as a result of the degradation of these materials during the composting process, would therefore be expected within the brown waste materials.

An overall decrease was seen for the copper amended brown waste materials but not the unamended, suggesting that the degradative processes within the composting materials were dependant upon the presence or absence of additional copper. However, as variation in the data for these materials was greater than the changes seen, they may well have not been significant.

Bands near 1640 cm^{-1} are known as the amide I band and correspond to the carbonyl stretching of secondary amides in proteins and polypeptides (Boyd *et. al.,* 1979). Cu^{2+} is known to form complexes with the peptide carbonyl group by displacing the proton from the peptide nitrogen through metal-nitrogen coordination. This leads to a shift to higher frequencies in the amide I band. Cu^{2+} is also known to form strong complexes with carboxylate ions (1620 cm^{-1}), which again leads to spectral shifts in the COO^- stretching vibrations (Baes and Bloom, 1989). However, no shifts were seen in this experiment, only indications of decreases in absorption (decreases being unproven owing to variations in the data) as a result of protein degradation.

Bands at 1240 cm^{-1} are associated with aromatics i.e. C-OH bonding of COOH phenols and tertiary alcohols (Paré *et. al.* 1999). No significant changes in absorption were seen, only a significant decrease (and hence, a decrease in aromatic compounds) between day 0 and day 318 for the APS. Increases in aromatics during composting are usual (Inbar *et. al.,* 1989), and clearly this has not happened here, indicating that the

materials were still immature. However, apparent decreases in absorption values at 1240 cm^{-1} relative to those at 1030 cm^{-1} (polysaccharides) are small for most of the brown waste materials and the EGS blend. Therefore the ratio of aromatic to aliphatic compounds seems to have increased over time, demonstrating that decomposition of the substrates was taking place.

Siebielec *et. al.* (2004), performed correlations between absorbance and metal content over the whole spectral range for metal contaminated soil samples using DRIFT spectroscopy and achieved an R^2 value of 0.80. The sum of extractable copper in fractions 1-5 of Tessier's scheme were found to negatively correlate with absorbance values for all frequencies. Values of R^2 ranged from -0.367 (p=0.05) for absorbance at 2850cm^{-1} to -0.470 (p=0.010) for absorbance at 1643cm^{-1}. Significant negative correlations were also found between total copper content as determined by XRF and all frequencies with the exception of 2850 cm^{-1}. As this means that increases in copper content (particularly extractable copper) leads to decreases in absorption at all frequencies, this suggests that the presence of copper may either be inhibiting absorption by the functional groups or, in some way, be promoting the degradation of the materials. In general, such metal complexes are poorly resolved in FTIR spectroscopy and require DRIFT spectroscopy to be seen (Baes and Bloom, 1989). It is therefore gratifying to achieve significant correlations between absorbance values and copper content with just FTIR.

3.5.4 Summary of Findings

- FTIR analysis of the samples has demonstrated clear reductions over time in both polysaccharide and proteinaceous contents of the materials.
- Clear increases in aromatic compounds have not been seen, but the ratio of aromatics to aliphatics appears to have increased in some materials.
- Correlation analysis found an association between copper content and functional groups suggesting that copper may either be inhibiting absorption by functional groups or may be promoting the degradation of the materials.

3.6 Water Extractions and Organic Matter Determination of Samples Taken at t=0 and t=318 days

3.6.1 Materials and Methods

Samples taken at t=0 and t=318 days which had been stored at -18°C were first defrosted and analysed for water and organic matter contents. Water extracts of the same samples were measured for pH; electrical conductivity (EC); NH_4^+-N and NO_3^-N; dissolved C; germination indices of cress seeds and absorbencies at 280nm, 465nm, and 665nm. Procedures for the analysis of the water extracts were first developed and perfected on water extracts of multipurpose compost.

1. Water and Organic Matter Contents. Defrosted samples were weighed out into pre-weighed evaporating basins and left at room temperature to air dry to constant weight. Basins were re-weighed, oven-dried overnight at 105˚C, cooled and then weighed again. Organic matter content was then determined by loss on ignition. The basins were placed overnight for ashing in a muffle furnace set at 550˚C. The difference in weight between oven drying and ashing at 550˚C was the organic matter content of the samples. Results were expressed as percentage organic matter content on an oven dry weight basis.

2. Water Extracts. Deionised water extracts were prepared by shaking samples with deionised water (1:10 dry weight/volume) at room

temperature for 2 hours (Chefetz *et al*. 1998). The supernatant was then decanted and centrifuged at 4,000 rpm to remove any suspended particles still present. After centrifugation the supernatant was then decanted using a transfer pipette to polypropylene containers and stored at 4˚C prior to analysis, although separate portions were set aside and stored separately under different conditions for ammonium, nitrate and dissolved carbon analysis. The water extracts were then measured for the parameters mentioned as follows:

pH

pH was measured using a Jenway 3510 pH meter, calibrated against commercially available buffer solutions of pH 4.0 and 7.0.

EC

Electrical conductivity was measured using a Paqualab 4071 electrical conductivity meter calibrated against a solution of KCl of conductivity 1413 µS cm^{-1}.

Ammonium and Nitrate Nitrogen (NH_4^+ and NO_3^-)

30ml of the supernatant were placed in a separate polypropylene tube and 1-2 drops of toluene were added to prevent losses of nitrogen by microbial activity. These tubes were then set aside for ammonium and nitrate analysis and stored at 4˚C prior to analysis. Extracts were analysed for ammonium and nitrate nitrogen by steam distillation (MAFF, 1986) on a Kjeltec 1030 autoanalyser. Briefly, 10ml of each sample

extract were dispensed into a 250ml test-tube, placed in the autoanalyser and steam distilled with 7ml of magnesium oxide suspension (17g in 100ml deionised water) to release the ammonium. The distillate was trapped in an indicator solution containing 1% Boric acid, methyl red and bromocresol green indicators and titration was then performed against 0.1N HCl until a pink coloured end point was reached. Once complete, the test-tube was removed from the analyser, approximately 1g of Devarda's alloy was then added, the tube returned to the autoanalyser and steam distilled once more. The Devarda's alloy reduced any nitrate present to ammonium, which, owing to the presence of the magnesium oxide was then distilled, trapped in the indicator solution and titrated against 0.1N HCl in the same manner as before. 10ml of ammonium and nitrate standards containing 0.14mgN/ml were prepared and analysed to check for recovery with titre-blank results of 1.000ml being equal to 100% recovery. Average recovery of the machine was 96.5% for the ammonium and 98.2% for the nitrate when the t = 0 days samples were analysed and 98.38% and 99.1% for the ammonium and nitrate samples when the t = 318 days samples were analysed.

Dissolved Carbon

Determination of dissolved carbon was first attempted using a LECO SC-144DR sulphur-carbon analyser (Leco Corporation, Michigan, USA). The machine was calibrated by pipetting water blanks and 0.1, 0.2…0.4 ml of a 1% solution of dried HEPES (containing 0.403 %C) onto a bed of COM-AID absorbent contained within a foil lined ceramic boat. The boats were

placed in the furnace of the machine and the resulting carbon released was measured by total combustion. Water extracts of the samples were analysed in the same manner as the blanks and standards. This approach failed for two reasons:

1. The machine was not found to be sensitive enough to measure the small quantities present in sample extracts.
2. More moisture was released than the machine could reasonably handle, causing the magnesium perchlorate filters present within the system to saturate and fail.

Another approach was needed and so, after various investigations, a procedure for the determination of chemical oxygen demand (COD) using a closed reflux method (APHA, 1985) was chosen. The principle behind the method is that most forms of organic carbon in aqueous samples can be oxidised by boiling with a mixture of chromic and sulphuric acids. During this method, samples are refluxed with an excess of acidified potassium dichromate and oxygen from the dichromate is consumed in proportion to the amount of dissolved organic carbon present in the sample. Any unreduced potassium dichromate is then measured by absorbance at 600nm in a spectrophotometer. Results for COD are therefore given as an oxygen equivalent of the organic matter content of the sample and not directly as mgl^{-1} of dissolved carbon. Prior to analysis, approximately 10ml of the centrifuged water extract was dispensed into glass universal bottles previously washed with 20% H_2SO_4. The samples

were acidified to <pH 2 with 1 drop of H_2SO_4, capped under nitrogen gas to prevent losses of carbon as CO_2 and stored in the dark. The spectrophotometer used was manufactured by Beckman (model No. DU 640) and all reagents used were Analar grade or better. Details of the whole procedure can be found in Appendix IX.

Germination Indices

Germination indices were carried out according to Keeling *et. al.* (1994) as a measure of phytotoxicity. 3ml of the water extracts were placed on Whatman No. 1 filter papers in 9mm plastic Petri dishes (Sarstedt). Twenty cress seeds (*Lepidum sativum* L.) were placed in each dish, the lids replaced and each dish sealed with laboratory film. 3 control plates containing 3ml of deionised water, a filter paper and 20 seeds were also set up. Dishes were randomly arranged on the bench top and kept at ambient laboratory temperatures for 4 days until there was extensive germination and growth within the control plates. Growth was checked at 4 days by the addition of 1ml of IMS and the plates stored at 4˚C prior to measuring the seedlings. The germination index was calculated using the following formula:

$$GI\ (\%) = \frac{\text{Total length of seedlings in test plates} \times 100}{\text{Total length of seedlings in control plates}}$$

Solutions producing a GI of < 100% were judged to be phytotoxic and those producing a GI of > 100% growth stimulating.

Optical Density

Extracts were filtered via a 0.2 μm membrane filter and measured at 280nm, 465nm and 665nm in a Beckman DU640 spectrophotometer using UV transparent cuvettes and diluting samples where appropriate to give absorbance values of < 1.0. E4/E6 ratios were calculated from absorbencies at 465nm and 665nm.

Data for the results of the organic matter analysis and also the analysis of the water extracts are presented in Figs. 3.23 and 3.24.

Spearman's rank correlation (adjusted for tied values) was performed in order to see if the various ways in which copper content and distribution had been determined could be associated with the various parameters measured. Correlation parameters chosen were: copper extractable in fraction 1 of the Tessier scheme, CRM adsorbable copper, copper extractable in fractions 1-5 of the Tessier scheme, total copper as determined by XRF, organic matter content results, all water extract data, total C, total N, C/N and total S. Full correlation matrices with their correlation coefficient values and their significances are given in Tables 1 and 2 of Appendix X. The software program Genstat (9^{th} Edition) was used for the calculations.

3.6.2. Results

Organic Matter Content

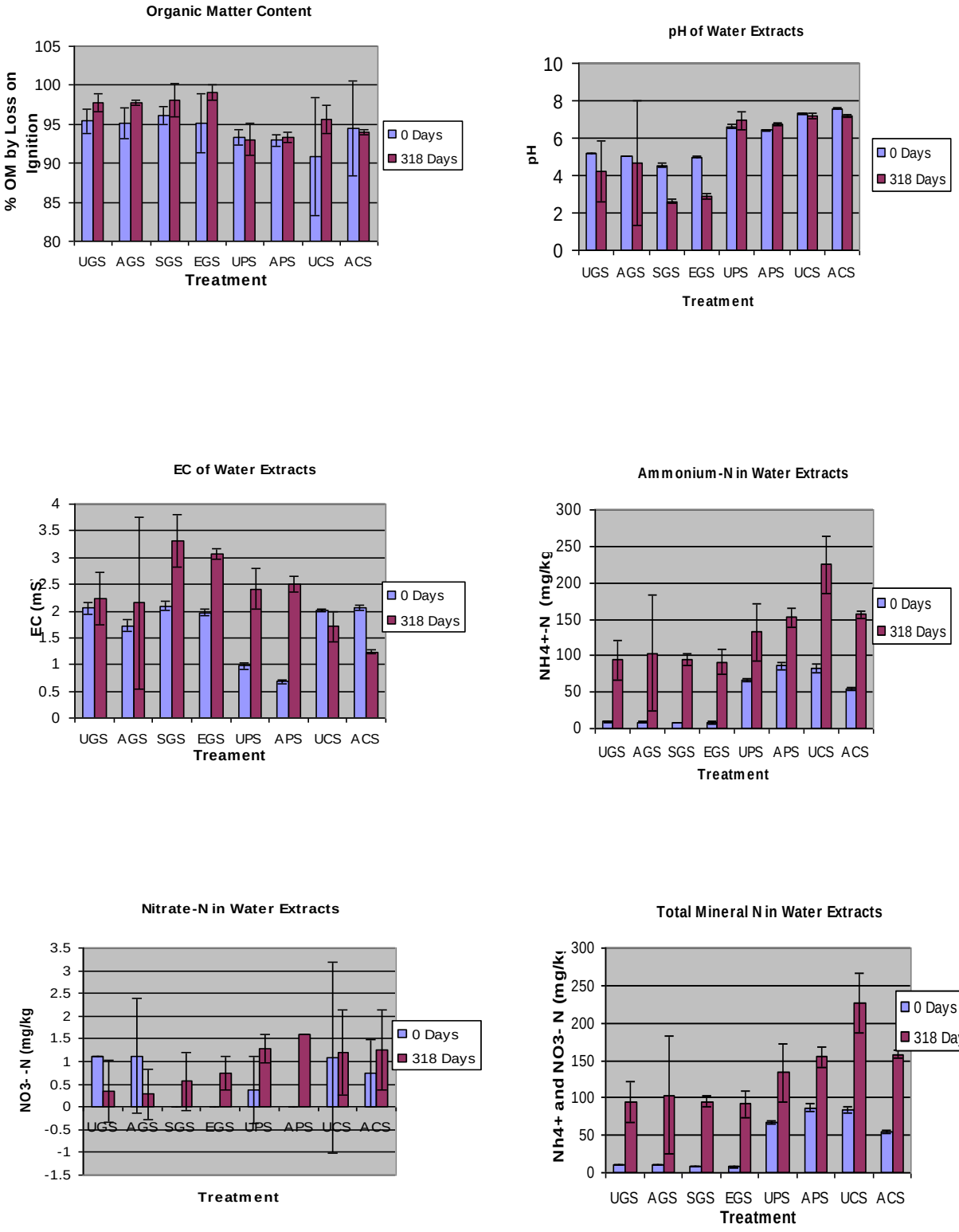


Fig. 3.23 Organic matter content of Samples t = 0 and 318 days; pH, EC, NH_4^+-N, NO_3^--N and Total Mineral N (Sum of NH_4^+-N, NO_3^- - N) of Water Extracts. Error bars are ± 95% Confidence Intervals.

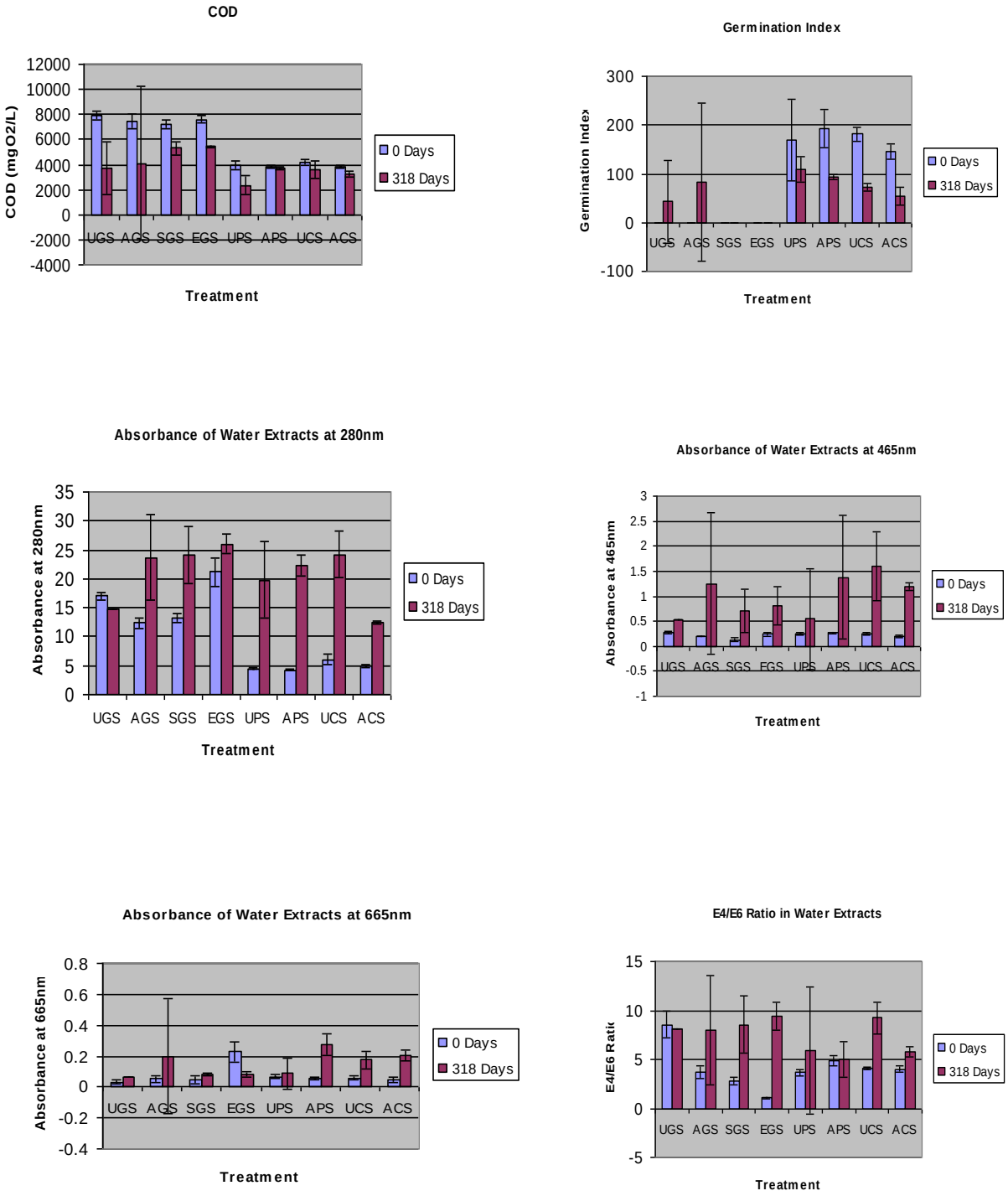


Fig. 3.24 Germination Index, Chemical Oxygen Demand (COD), Absorbencies at 280nm, 465nm and 665nm, E4/E6 Ratio of Water Extracts. Error bars are ± 95% Confidence Intervals.

Changes in organic matter content were not readily apparent over the course of the experiment. Only in the UGS, SGS and EGS blends did the data suggest that increases in organic matter had occurred. However, only with the AGS was this significant. Only among the 4 treatments of the green waste blend was organic matter content significantly correlated with copper content. Here, organic matter content was significantly positively correlated with total copper as determined by XRF.

Organic matter content was significantly negatively correlated with pH and COD and significantly positively correlated with EC in the various grass/sawdust treatments and negatively correlated with germination index values within the UGS, AGS and all the treatments of the brown waste blends.

pH

pH values for the grass/sawdust materials all remained very acidic which suggests the occurrence of anaerobic activity. pH values for the brown waste materials were closer to neutral. SGS, EGS, and ACS treatments appeared to decrease in pH during composting, while the APS treatment increased. Other blends and treatments did not seem to change during the experiment. This may have been due to wide variations within some of the data, or maybe that changes were simply missed owing to the fact that only 3 sampling times were used. pH was significantly negatively associated with copper in the 4 treatments of the green waste blend as

determined by chelating resin membrane, total sequentially extractable copper and total copper by XRF. However, only a significant negative correlation was seen with CRM extractable copper within the unamended and copper acetate treatments of the 3 blends.

Electrical Conductivity (EC)

Increases in EC were seen within the SGS and EGS and within both the UPS and APS blends. The ACS was seen to decrease in EC during composting but no other materials demonstrated any measurable changes.

Ammonium and Nitrate Nitrogen

Ammonium nitrogen was seen to have increased substantially in all materials by the end of the composting period. Nitrate nitrogen did not change in any measurable way. However, this is likely to be due to the fact that only very small quantities were found and variation among replicates was very high. Consequently, trends in the sum of the two forms of extractable nitrogen (ammonium plus nitrate), followed the same pattern as that for ammonium nitrogen by itself.

COD

COD decreased over the course of the experiment in both the UGS and UPS materials, but did not decrease in the UCS. Among the copper

amended treatments, COD was seen to decrease in the SGS and EGS and also within the ACS materials. Large variation within the data of the AGS meant that any changes that occurred could not be demonstrated.

Germination Index

At 0 days, all the grass/sawdust treatments, including the unamended material were extremely phytotoxic. Of the unamended replicates, only 3 seeds germinated and of the copper sulphate treated replicates, only 2 seeds germinated. This resulted in these treatments having a GI of <0.1% of the control. No seeds at all were seen to germinate in the AGS and EGS treatments and so GI values for these were 0%. By the end of the experiment, GI had increased in the UGS and AGS materials but they were still appeared to be phytotoxic. However, large variation in the data meant that this may not have been the case. Unamended brown waste materials were found to be growth stimulating with GI values exceeding those of the control plates. During the course of composting these values decreased, and GI values became lower than the controls (i.e. <100%). However, variation within the data of the UPS on both sampling dates, suggest that GI values are probably not significantly different from 100%. The only clear difference between treatments was between the UCS and ACS at t = 0 days. Here, the ACS had a significantly lower GI than the UCS. Clearly the additional copper had a statistically significant phytotoxic effect.

Absorbances at 280nm, 465nm, 665nm and E4/E6 ratios

With the exception of the UGS, absorbances of all the water extracts at 280nm increase during the composting process. At 465nm the pattern is almost the same, although absorbances at this wavelength appear to increase for all the materials, including the UGS. However, this cannot be said with certainty for the AGS and SGS and both treatments of the pig slurry/sawdust materials at t = 318 days as large variations were found in the data. Problems with large variation in the data for the AGS as well as the UPS, also meant that increases seen during composting may not have been real. Absorbances at 665nm for the other brown waste treatments appear to increase over time, whilst those for the EGS decrease. Owing to loss of samples, a 95% confidence interval for the UGS at t = 318 days could not be established. E4/E6 ratios also increased for most treatments. However, large variation within the data for absorbances at 465nm meant that large variations were also seen for the AGS as well as both pig slurry/sawdust treatments. These increases may therefore not be real.

Spearman's Rank Correlation Analysis – Grass/Sawdust Only Blends

Copper extracted in fraction 1 of the Tessier sequential extraction scheme was very highly significantly negatively correlated ($p \leq 0.001$) with ammonium nitrogen, ammonium + nitrate nitrogen, and C/N ratios;

highly significantly negatively correlated ($p \leq 0.010$) with nitrate nitrogen and total organic nitrogen; and significantly negatively correlated ($p \leq 0.050$) with absorbances at 465nm and total organic carbon content.

CRM extractable copper was very highly significantly positively correlated ($p \leq 0.001$) with absorbances at 665nm; highly significantly positively correlated ($p \leq 0.010$) with absorbances at 465nm and significantly negatively correlated ($p \leq 0.050$) with pH and GI values.

Copper in the sum of fractions 1 to 5 of the Tessier sequential extraction scheme (i.e. total extractable copper) was very highly significantly positively correlated ($p \leq 0.001$) with total copper as determined by XRF; very highly significantly negatively correlated ($p \leq 0.001$) with pH values; significantly positively correlated ($p \leq 0.050$) with both EC and E4/E6 ratios and significantly negatively correlated ($p \leq 0.050$) with COD.

Total copper as determined by XRF very highly significantly positively correlated ($p \leq 0.001$) with copper in the sum of fractions 1 to 5 of Tessier's sequential extraction scheme (i.e. total extractable copper); very highly significantly negatively correlated ($p \leq 0.001$) with pH values; highly significantly positively correlated ($p \leq 0.050$) with both EC and total sulphur content; significantly negatively correlated ($p \leq 0.050$) with COD and positively correlated ($p \leq 0.050$) with organic matter content.

Spearman's Rank Correlation Analysis – Unamended and Copper Acetate Treated Blends

Copper in fraction 1 of the Tessier sequential extraction scheme was very highly significantly positively correlated ($p \leq 0.001$) with copper determined in the sum of fractions 1-5 of the Tessier scheme and also CRM extractable copper. It was positively significantly correlated ($p \leq 0.050$) with total copper as determined by XRF.

Chelating resin membrane extractable copper was very highly significantly positively correlated ($p \leq 0.001$) with copper determined in the fraction 1 and the sum of fractions 1-5 of the Tessier scheme; highly significantly positively correlated ($p \leq 0.010$) with total copper as determined by XRF and significantly negatively correlated ($p \leq 0.050$) with pH values.

Copper extracted in the sum of fractions 1-5 of the Tessier sequential extraction scheme was very highly significantly positively correlated ($p \leq 0.001$) with copper determined in fraction 1 of the Tessier scheme, CRM extractable copper and total copper as determined by XRF. It was positively significantly correlated ($p \leq 0.050$) with absorbencies at 665nm and negatively significantly correlated ($p \leq 0.050$) with COD.

Total copper by XRF was very highly significantly positively correlated ($p \leq 0.001$) with copper determined in the sum of fractions 1-5 of the Tessier

sequential extraction scheme; highly significantly positively correlated ($p \leq 0.010$) with CRM extractable copper; significantly positively correlated ($p \leq 0.050$) with copper in fraction 1 of the Tessier scheme, absorbencies at 665nm and total sulphur. It was negatively significantly correlated ($p \leq 0.050$) with COD.

3.6.3. Discussion

Compost stability and maturity are frequently used terms within the literature. It has been noted that they are sometimes used interchangeably (Wu *et. al.* 2000). However, stability generally refers to the extent to which microbial activity continues to degrade the substrates in question, whilst maturity refers to the quality of the end product and its ability to support plant growth owing to the degree of decomposition of phytotoxic organic compounds produced during the active phase of composting (Wu *et. al.* 2000; Zmora-Nahum *et. al.* 2005). However, composted material may be mature and yet still be phytotoxic owing to the presence of soluble salts or high heavy metal concentrations (Wu *et. al.* 2000). This part of the study sought to establish whether a) the composting process employed produced mature products that supported plant growth or whether b) the presence of copper within the composting systems inhibited degradation of the materials and/or inhibited plant growth (and hence the development of maturity).

Assessment of compost maturity is often achieved by evaluation of chemical parameters (Zmora-Nahum *et. al.* 2005). Organic matter content and the various parameters measured here within the water extracts of the material are often used as indicators of compost maturity (Mathur *et. al.* 1993). Organic matter contents usually decrease with time as material degrades and is lost as CO_2, CH_4 and N_2O and NH_3 (Kuo *et. al.*, 2004). COD has also been seen to decrease with composting time, regardless of the initial substrates used, and correlate with absorbance at 465nm (Zmora-Nahum, 2005). However, with the exception of a small increase in the AGS materials, no measurable changes in the organic matter contents of the materials were seen. This general lack of change is reflected in the various negative and positive correlations found between pH, COD, GI and EC, all of which demonstrate that the materials were both unstable and immature.

pH is often seen to decrease during the initial mesophilic phase of composting (Gao *et. al.* 2005) but then recover and increase until it stabilises around neutral. As pH was only measured at 0 and 318 days, any decreases that may have occurred during the mesophilic phase were missed. This would explain why few changes were seen in the brown waste and the unamended and copper acetate treated green waste materials. Large decreases in pH were seen in the SGS materials, perhaps as a result of the formation of sulphuric acid. EGS materials also decreased in pH during composting, despite increases in ammonium – N which are normally associated with the proteolysis of amino acids,

proteins etc. (Ayuso *et. al.* 1996) and concurrent increases in pH. It is possible that the decreases seen in pH may be a result of lengthy decomposition of the EDTA ligands. Large amounts of ammonium may have been released, but in this situation the breakdown of the EDTA ligand would also result in the release of acetic acid, thus lowering the pH. pH was found to be significantly negatively correlated with copper in the grass/sawdust materials (all treatments), as determined using chelating resin membrane (CRM); total copper by XRF and total extractable copper as defined by the sum of fractions 1 to 5 of the Tessier scheme. Within all three of the unamended and copper acetate treated materials, only CRM copper was seen to be related to pH. Significant negative correlations of extractable copper with pH have also been seen by other workers (Gao *et. al.* 2005; Amir *et. al.* 2005). This is due to copper existing in cationic (and hence soluble) forms e.g. Cu^{2+} and $Cu_2(OH)_2^{2+}$ at low pH values (Szymanski *et. al*. 2005).

Significant negative correlations have been seen in all materials between dissolved organic carbon (as measured by COD) and total extractable copper within the five fractions of the Tessier scheme. Changes in water soluble carbon have been reported as closely following changes in water soluble copper (Hsu and Lo, 2001). It is therefore surprising that significant correlations were not also seen for copper extracted in fraction 1 of the sequential extraction scheme as they were for Gao *et. al*. (2005). Decreases in DOC during composting are usually reported (Inbar *et. al.* 1993) and have been attributed to the soluble sugars and amino acids

being degraded by microbes and converted to CO_2 (Chefetz *et. al.*, 1998). COD was seen to decrease in the nearly all of the materials. The only exceptions to this was the APS blend, leading to the conclusion that the presence of copper acetate in the pig slurry based blend inhibited microbial breakdown of dissolved carbon compounds. DOC in the UCS also appeared to decrease, however variation within the data meant that this was unproven.

Optical properties have been used as rapid indicators of DOC with highly significant positive correlations having been observed between absorbances at 465nm and 665nm (and hence the E4/E6 ratio) and DOC content (Inbar *et. al.* 1993). In these samples, significant negative correlations were seen between absorbances at 465nm (all materials) and 665nm (all unamended and copper acetate treated materials). No correlations were seen between COD and E4/E6 ratios although E4/E6 ratios appeared to largely increase with time. In terms of predicting copper content, CRM extractable copper was significantly positively correlated with absorbance at 280nm and significantly negatively correlated for the grass/sawdust materials. Total extractable copper by the Tessier scheme was significantly positively correlated with E4/E6 ratios; but copper in fraction 1 of the scheme was significantly negatively correlated with E4/E6 ratios for the same samples. Absorbances at 665nm, like the COD values, were significantly negatively correlated with total extractable copper and total copper by XRF for all the unamended and copper acetate treated blends. However, copper extracted from the

unamended and copper acetate treated blends by fraction 1 and by CRM was not correlated with any of the optical properties measured. Trends are not often clear (Lasiridi *et. al.* 2000) and Chen *et. al.* (1977) found that high E4/E6 ratios were related to low molecular weight compounds and *vice versa*. As E4/E6 ratios are often reported as increasing during the composting process (as also found in this experiment) this suggests that there are fewer aromatic structures in the end product than at the start (Lasiridi *et. al.* 2000). However, it has been reported that “young” forms of humic acids separated from decomposting plant residues were of a higher molecular weight at the start of composting than at the end (Riffaldi *et. al.,* 1983; Zhigunov and Simakov, 1977). If this is the case, then the materials under investigation in this project may have followed a similar degradative pattern. E4/E6 ratios do not always increase. Ratios in wood shavings have been seen to decrease, whilst those in peat moss and sawdust remained steady (N’Dayegamiye and Isfan, 1991).

Phytotoxicity as measured by germination indices is seen with fresh substrates (Ayuso *et. al.* 1996) and is due to the presence of phytotoxic compounds and competition for O_2 (Inbar *et.al.* 1993). At the start of the experiment, all brown waste blends were growth promoting, their GI values > 100%. However, during the composting process, GI values decreased and the materials became growth inhibiting. This may be due to increases in ammonium levels, as ammonium has been shown to have a phytotoxic effect (Murillo *et. al.* 1995). However, no significant correlation between GI and ammonium was found for these materials.

Significant correlations were seen with pH but this took into account pH values for the grass/sawdust materials and pH values of the brown waste blends which changed very little. No correlations were seen between GI and copper content.

Green waste blends were extremely phytotoxic. No seeds germinated in the t = 0 days samples for any of the treatments and by 318 days, only the UGS and AGS materials had increased their GI values with cress seeds in the extracts of the SGS and EGS treatments failing to germinate. GI values for the green waste materials correlated negatively with CRM extractable copper and absorbance at 280nm but with no other parameter. In fact, GI did not correlate significantly with any other copper determination, either for the green waste or brown waste materials. In this instance GI is therefore a relatively poor indicator of copper content. However, given that there was a significant negative correlation with CRM copper, it might be concluded that at least for green waste based composted materials, CRM adsorbable copper is a useful way to determine phytoavailable copper.

Significant negative correlations were seen between the ammonium; nitrate; sum of ammonium + nitrate contents of the green waste blends; total nitrogen and copper extracted in fraction 1 of the Tessier sequential extraction scheme. As these are some of the easiest parameters to measure, it must be concluded that these would be good, practical indicators for predicting extractable copper in these types of materials.

3.6.3. Summary of Findings.

- Lack of change in overall organic matter contents of the samples demonstrates a lack of maturity of the materials.
- Copper in the materials investigated has been seen to correlate with pH, COD, GI and optical properties of the materials' water extracts.
- Correlations between copper and these parameters depend on the form in which copper is measured. GI values were a poor predictor of copper content in these materials.
- Decreases in COD and increases in E4/E6 ratios illustrate the maturing process of the material. However, increases in ammonium for most materials suggest that the materials are still unstable and immature.
- Decreases in pH values for the SGS and EGS materials are probably a result of the nature of the copper treatment.
- Easily exchangeable copper in green waste blends was successfully correlated with a range of easily determined parameters. This may therefore be a potentially suitable approach for predicting available copper in these types of treated materials.

Chapter 4

Summary and Conclusions

Composting can be an effective way of dealing with biodegradeable wastes and forms part of the UK Government's *'Waste Strategy 2000'* (DETR, 2000a) initiative. However, problems can arise if these wastes are contaminated with heavy metals and the concentrating effect of the composting process can magnify such problems. Plant availability of heavy metals such as copper is affected by their chemical forms and speciation rather than merely the total amount present (Quevauviller *et. al.,* 1997). Therefore, this project set out to determine how, using sequential extraction techniques, the composting process affected the speciation of copper in a variety of biodegradeable wastes. Results gained from these techniques were compared with copper as determined by a chelating resin membrane approach and also total copper by XRF. Composting materials were characterised for total C, N, S, functional groups (by FTIR), organic matter and various water extractable parameters (pH; NH_4^+ and NO_3^- - N; DOC; EC; absorbencies at 280nm, 465nm, and 665nm and phytotoxicity by determination of the germination index of cress seeds).

Composting of a mixture of grass clippings and sawdust originating from tanalised timber was carried out (Chapter 2). The starting materials were

either unamended or were treated with differing amounts of soluble copper, using a copper acetate solution, and then composted. Results showed that at the start of the experiment over 80% of the copper present in the unamended materials occurred in forms not immediately available for plant uptake. However, composting processes enabled the release of this copper which then, over time, would have become more bioavailable. The end product contained over 500 $mgkg^{-1}$ of total extractable copper and would therefore fail to meet the British Standards Institute PAS 100 Specification for Composted Materials. Where materials were amended with a copper solution the copper was seen to quickly react with the substrate such that large amounts of copper were detectable in all fractions except the residual one. The data indicated that, over time, copper moves from the very easily extractable fractions, the reducible and also the residual fractions to the EDTA extractable fraction, thought to determine organically complexed / chelatable metals (Amir, 2005). This continued until equilibrium was reached and then the water and calcium nitrate extractable forms appeared to hold the excess. Copper as determined by these extracts would be available for plant uptake. These changes would not have been detected on the basis of a measurement of total copper alone; especially as the sum total of copper in all the fractions (total extractable copper) established that there were no changes in the overall amount of copper present in the treated materials.

Three different organic wastes (grass/sawdust, pig slurry/sawdust and sewage sludge cake/sawdust) to which copper had been added as

copper acetate, sulphate or EDTA, were then composted in the laboratory. Samples were taken at 0, 105 and 318 days and subjected to the range of analyses mentioned. Sequential extractions demonstrated clear changes in copper distribution amongst various fractions within the materials. Release of copper which had already been present in the materials moved from the oxidisable to easily extractable (and hence potentially phytoavailable) fractions in the control samples. In copper amended materials transfer from available fractions to less available fractions was seen. Transformations of easily extractable to less available copper within copper EDTA treated materials were slowest of all. Blend type determined the initial amount of copper extractable in fraction 1, which in turn determined the rate at which it was transformed into other extractable fractions. Comparisons made between the performance of two sequential extraction schemes (BCR and Tessier *et. al.* schemes) showed similar trends in metal distribution although amounts and proportions varied. In most cases the Tessier scheme was found to be more efficient at extracting copper than the BCR scheme. This was the first time that such a comparison had been made on such materials.

CRM determined copper correlated strongly and highly significantly with copper from fraction 1 and also the sum of fractions 1 and 2 of the Tessier scheme for all the untreated and copper acetate amended materials, though changes over time did not correspond well. It also correlated with GI values in the grass/sawdust blended materials, though sequentially extractable copper did not. The procedure was limited by the

length of time taken to determine the field capacity of the material as well as possible lack of homogeneity of the materials. However, if these limitations could be overcome, this might be a viable approach to the routine determination of phytoavailable copper in these types of materials.

Other results also showed that changes (and therefore degradation of the materials) were taking place. FTIR analysis of the samples demonstrated clear reductions over time in both polysaccharide and proteinaceous contents of the materials. And while clear increases in aromatic compounds have not been seen, the ratio of aromatics to aliphatics appears to have increased in some materials. Correlation analysis found an association between copper content and functional groups suggesting that copper may either be inhibiting IR absorption by functional groups or may be promoting the degradation of the materials.

Decreases in COD, C/N ratios and increases in E4/E6 ratios were also seen and illustrated the maturing process of the materials. However, other results clearly demonstrated that they were still unstable and immature. C/N ratios of the end products still exceeded 20:1; the ammonium content in most materials were seen to increase and there was a lack of overall change in the organic matter content of the materials. Such slow decomposition was attributed to the high lignin

content of the materials and may have accounted for the apparent increases in total C, N and S that were seen.

It was unfortunate that, for these materials, sequentially extractable copper also proved to be a poor predictor of GI values. However, in the green waste blends, easily extractable copper was successfully correlated with a range of easily determined parameters (Appendix X). These may therefore be a potentially suitable approach for predicting available copper in these types of treated materials.

Composting carried out as part of this project was small and at the laboratory scale. This approach had its limitations, particularly as the blends under investigation were of a lignaceous nature. Decomposition in both trials was slow, even where pig slurry and sewage sludge cake were used as co-composting substrates and even after 318 days the materials were still immature. If further trials were to be conducted using lignaceous materials then they should be at a much larger scale. Nonetheless, copper redistribution in all materials was clearly demonstrated, showing that as a process, composting is valuable in immobilising copper in situations/materials where it is initially phytoavailable and at phytotoxic levels. Where copper was initially present largely in organically bound forms e.g. the sewage sludge cake used here, composting processes released the metal to fractions which would be phytoavailable. Sequential extraction analyses were useful in revealing this. However, the three

different schemes used were all found to be very time-consuming procedures.

Use of chelating resin membranes is a novel approach which is much faster (once field capacity values have been determined) and, despite variation seen in the data, shows promise. Most of the work to date has centred on the area of soil nutrition and there have been no reports of use in composting materials. It would therefore be very interesting if future work would develop the chelating resin approach for use in these products. Resin use could be in either membrane or pellet form and a range of macro and micronutrients studied. Use *in situ* within large scale composting windrows as well as developing reliable and commercially useful lab-scale systems for resin analysis would present a variety of challenges. Assessment of the optimum size of pellet/membrane in relation to the particle size of the materials investigated should be a focus, as well as determining the extent to which organic compounds interfere/enhance metal adsorption by the resin.

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Appendix I

Limiting the availability and toxicity of copper in waste-derived composts.

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Summary

Copper is a common contaminant of organic wastes and is also toxic to soil organisms and plants at relatively low concentrations. The purpose of this project is to determine the effect of composting process parameters (time, degree of aeration, moisture content, C:N ratio) on the chemical forms of copper in a green waste consisting of grass and wood shavings. It is shown that significant changes do take place over the composting period. These changes offer the potential to modify process conditions and reduce plant-available and toxic forms of the metal.

Introduction

Sustainable human social systems require that waste products are utilised wherever possible and raw material use is kept to a minimum. Organic wastes from kitchens, parks and gardens and sewage sludges are examples of waste products which are difficult to minimise and may be contaminated with industrial wastes containing toxic elements and compounds. However, organic wastes are, in principle, the simplest wastes to recycle because they can be composted and returned to the soil. Composts can substitute for fertilisers in crop production (Sikora et al, 1998) and help maintain soil fertility and increase resistance to water and wind erosion (De Vos et al, 1996). They can also act as substitutes for peat in horticultural growing media (Rainbow and Wilson, 1998).

Heavy metals (Cu, Ni, Pb, Zn, Cd, Hg) are common components of both industrial and domestic waste streams, being found in a range of cosmetic and medicinal products, batteries and discharges from metal-working processes, and are therefore found in raised concentrations in composted wastes, where the contamination problem is made worse by two-fold concentration by the aerobic digestion process. Most heavy metals, when present in low quantities in soil, act as essential micronutrients, being components of enzymes and taking part in a number of plant metabolic pathways. However, they are toxic to plants at higher concentrations, causing a range of symptoms including chlorosis and stunting of roots and shoots. The mean soil concentration of these metals, and the maxima generally tolerated by plants, are shown in table 1. However, plants can tolerate higher heavy metal concentrations than humans and other animals, and some plants are hyperaccumulaters of metals, for example, cruciferous plants can accumulate Zn to render them less palatable to potential predators (Morel, 1998). In addition to being a threat to human health when in the food chain, heavy metals can be extremely toxic to certain soil microorganisms (Hattori, 1992). Copper is a very common contaminant of waste and can abolish the growth of nitrogen-fixing bacteria at low concentrations (Keeling and Cater, 1998). It is therefore important that steps are taken to reduce the damaging effects of common metals in soil applied composts and waste products.

Increasing quantities of metal contaminated sewage sludge and composts are likely to be produced over the next few years, particularly as sea-dumping of sewage sludge is banned. and methods of limiting metal toxicity in contaminated organic wastes would be useful, since only readily plant-available fractions are likely to be toxic or enter the food chain. The quantity of plant-available metal could be manipulated by controlling composting conditions, for example, degree of aeration, C:N ratio and moisture content.

The purpose of this research was to determine how composting of a mixed waste derived from grass clippings and wood shavings could influence the chemical forms and plant availability of copper. Copper was chosen for specific study as it is a common contaminant in waste and is especially toxic to the soil microflora.

Table 1. Concentrations of heavy metals in UK soils, and limits for plant growth.

Metal	Mean concentration In UK soils (mg/kg)	Max. tolerable concentration for plant growth (mg/kg)
Cadmium (Cd)	0.8	50
Copper (Cu)	23	100
Lead (Pb)	74	300
Zinc (Zn)	97	400
Nickel (Ni)	25	100
Mercury (Hg)	-	50

Current legislation sets limits of heavy metals in waste materials according to their total metal content. Bioavailability though is dependent upon the metal speciation which arises from the competition among different complexing species for these metal ions (Massiani and Domeizel 1996 and Charlet and Manceau 1992). There are therefore moves within the EU to change these limits to take bioavailability into account. Adsorptive surfaces take part in a number of different reactions - adsorption, coprecipitation and surface precipitation reactions - and are responsible for the 'immobilisation' of metals whilst reactions with mineral surfaces may actually 'mobilize' metals (Charlet and Manceau 1992). The use of sequential extraction techniques in speciation studies is long-established and a variety of procedures have been developed. These provide operational information as to the likely processes occurring within a range of different materials including composts. This approach will not therefore provide much in the way of very detailed specifics regarding chemical speciation. For this, other, more non-invasive techniques must be employed.

Methods

Compost Preparation

The compost blend was prepared using grass clippings and pine sawdust at a ratio of 1:1.5 to give an estimated C:N ratio of 41:1. All large particles within the clippings (twigs, leaves and other foreign materials) were removed and the sawdust screened to pass through a 5.6mm mesh sieve. 100g of the blend were weighed out into 20 wide-necked low density polyethylene bottles.

In order to 'contaminate' the composting substrate a 5000 mgl^{-1} solution of copper acetate was prepared (BDH, Analar grade) and added to the bottles at the following rates: 0 copper (30ml water only); 50mg copper (10ml of acetate + 20ml water); 100mg copper (20ml acetate + 10ml water) and 150mg copper (30ml acetate only). The acetate salt was chosen because this is known to be water soluble. The contents of each bottle were thoroughly mixed and 5 bottles per treatment level were prepared. All compost samples had an initial moisture content of 64%.

Under normal composting conditions temperatures at the centre of a pile or windrow can exceed 70°C and be maintained for significant periods of time due to the insulating properties of the surrounding material. Weekly turning ensures that all the material attains this temperature at some point over the composting period and is thus sanitized. In a laboratory experiment, much smaller quantities are used making these temperatures impossible to attain. A temperature profile which mimics that of a large windrow was therefore applied artificially using an incubator. The bottles (lids on) were placed in an incubator: 0 to 7 days at 35°C; 7-21 days at 50°C; 21-37 days at 65°C and then 37- 238 days at 55°C. Bottles were shaken and aerated every 2-5 days. One bottle per treatment level was removed at 0, 7, 21, 179 and 238 days and frozen until required for analysis. On day 31 it was noted that 5 of the bottles (one at each level of copper) were not degrading as well as the others. Humification appeared to have stopped and there was a distinct alcoholic smell. This was probably due to a lack of headspace in the vessel making aeration difficult and leading to anaerobic conditions. To remedy the situation the contents of these bottles were transfered to larger containers and not sampled until day 238.

Solutions were prepared using deionised water and all plastic containers were soaked overnight in 69% nitric acid (BDH, Analar grade) to remove any contamination that may have existed as a result of previous work.

Analyses were carried out in triplicate. The samples were defrosted, dried at 80°C for 2 days and then ground (Cyclotec mill, Tecator, Sweden) to pass a 1mm sieve. 2g of the material were weighed out to within 0.001g into 60ml polysulphone centrifuge tubes (Fraser and Lum 1983), (Nalgene oak ridge with sealing caps) and copper extracted sequentially as follows:

1. Water soluble copper - 16 hours at room temperature
2. Neutral salt extractable/ exchangeable copper - 0.5M calcium nitrate solution for 16 hours at room temperature (Miller et al, 1986).
3. Surface oxide and carbonate bound copper - 1.0M sodium acetate solution at pH 5.0. 5 hours at room temperature (Fraser and Lum, 1983).
4. Fe and Mn oxide bound copper - 0.04M hydroxylammonium chloride in 25% acetic acid. 6 hours at 90°C (Fraser and Lum, 1983)
5. Organically bound copper - 0.1M potassium di-phosphate at pH 10. 16 hours at room temperature (Bascomb, 1968).
6. Carbonate occluded; chelate extractable copper - 0.1M EDTA. 16 hours at room temperature (Miller et al, 1986).
7. Residual / non-extractable copper - remaining sample ashed at 500°C and then digested in 20ml of 69% nitric acid.

Volumes (30 ml) of each extracting solution were used. Sample tubes were shaken on a Gallenkamp horizontal shaker for the required period of time with the exception of extraction number 4 when they were heated in a water bath and occasionally agitated by hand. At the end of each of the extraction periods the tubes were centrifuged at 15,000 rpm in a Beckman Avanti 30 centrifuge at 4°C for 30 minutes and the supernatants decanted. The decanted supernatants were filtered via a Whatman 541 filter paper (MAFF, 1986) and then stored frozen in 50ml HDPE pots with polypropylene screw on lids. Prior to freezing, supernatants from the water and calcium nitrate extractions were acidified to pH<2. Copper in each fraction was detemined on a Smith-Hieftje 1000 atomic absorption spectrophotometer. Each sample underwent a deionized water wash between extractions (Miller et al, 1986). Samples were shaken briefly by hand in 20ml deionized water and then centrifuged at 15,000 rpm for 15 minutes. The resulting supernatant was discarded.

Separate whole samples were analysed for total copper by a standard digestion in concentrated nitric acid (BDH, Analar grade) and subsequent analysis by atomic absorption spectrometry.

Results

Data are presented in both $mgkg^{-1}$ and as percent of the total copper extracted from each sample in tables 1 and 2 respectively. Figure 1 shows graphically the changes in copper partitioning over the composting period for the 0 mg and 150 mg treatments.

Table 2. Copper extracted from each sample (mgkg^{-1}; nd = not detected)

Treatment mg Cu/ 100g fresh material	Time/ days	Fractions mg Cu/ kg air dry compost									
		Water	Calcium Nitrate	Sodium Acetate	Hydroxyl-ammonium chloride	K4P2O7	EDTA	Residual	Total extracted	Total Cu mgkg	% Cu Recovered
0	0	6.593	nd	3.97	1.14	0.74	45.11	434.2	491.3	450.2	109.1
	7	26.99	2.18	12.08	3.80	8.63	60.26	310.5	424.4	428.8	98.98
	21	14.58	0.79	7.94	2.98	11.04	86.91	379.0	503.2	585.8	85.91
	179	15.48	Nd	13.11	21.10	27.79	191.1	318.4	585.8	437.9	133.8
	238	80.38	22.21	34.55	111.8	38.71	139.2	114.7	541.5	418.3	129.5
50	0	169	14.19	345.8	40.16	93.68	297.9	249.9	1211	1090	11.1
	7	270.2	23.43	241.3	29.86	138.5	410.8	366.2	1480	1094	135.3
	21	245	22.51	281.8	96.83	131.1	478.1	339.1	1594	1169	136.4
	179	225.2	38.71	109.8	326.75	141.9	370.3	236.8	1449	1079	134.3
	238	316.8	96.47	99.56	363.74	89.35	289.3	194.0	1449	1088	133.2
100	0	509.3	75.42	787.2	243.5	279.3	276.8	487.5	2659	1944	136.8
	7	438.7	54.24	579.1	238.7	336.4	339.3	391.5	2378	1922	123.7
	21	530.3	58.41	510.7	240.3	324.9	408.4	380.4	2454	1860	131.9
	179	168.8	7.10	278.9	516.9	423.3	712.3	407.1	2514	2097	119.9
	238	555.6	335.9	79.72	428.3	91.27	573.5	266.7	2331	1799	130.0
150	0	655.9	119.7	1128	258.2	434.9	242.5	500.4	3339	2776	120.3
	7	799.9	111.7	991.4	276.3	531.0	320.3	326.7	3357	2793	120.2
	21	642.6	87.56	917.3	343.2	530.9	370.5	388.7	3281	2681	122.4
	179	375.1	156.7	590.1	888.8	480.0	595.6	386.3	3473	2590	134.1
	238	456.6	753.4	153.4	504.2	167.9	607.0	303.2	2946	2375	124.0

Table 3. Copper extracted (% of total)

Treatment mg Cu/ 100g fresh material	Time/ days	Fractions % of total copper extracted						
		Water	Calcium Nitrate	Sodium Acetate	Hydroxyl-ammonium chloride	K4P2O7	EDTA	Residual
0	0	1.34	0	0.81	0.23	0.15	9.18	88.38
	7	6.36	0.51	2.85	0.90	2.03	14.20	73.15
	21	2.90	0.16	1.58	0.59	2.19	17.27	75.31
	179	2.64	0	2.24	3.60	4.75	32.62	54.35
	238	14.84	4.1	6.38	20.65	7.15	25.70	21.18
50	0	13.96	1.17	28.56	3.32	7.74	24.61	20.64
	7	18.25	1.58	16.30	2.02	9.35	27.76	24.74
	21	15.37	1.41	17.67	6.07	8.22	29.99	21.27
	179	15.53	2.67	7.57	22.54	9.79	25.54	16.34
	238	21.86	6.66	6.87	25.10	6.17	19.96	13.39
100	0	19.15	2.83	29.60	9.16	10.50	10.41	18.33
	7	18.45	2.28	24.35	10.04	14.15	14.27	16.47
	21	21.61	2.38	20.81	9.79	13.25	16.65	15.51
	179	6.71	0.28	11.09	20.56	16.83	28.33	16.19
	238	23.83	14.4	3.42	18.38	3.92	24.60	11.44
150	0	19.64	3.58	33.77	7.73	13.02	7.26	14.99
	7	23.83	3.33	29.53	8.23	15.82	9.54	9.73
	21	19.59	2.67	27.96	10.46	16.18	11.29	11.85
	179	10.80	4.51	16.99	25.60	13.82	17.15	11.12
	238	15.50	25.58	5.21	17.12	5.70	20.61	10.29

Water extracts - free copper ions in solution

With the exception of the 0 Cu treatment, which showed a marked increase in the levels of water extractable copper between 179 and 238 days, no clear trends were seen.

1.0M sodium acetate extracts - surface oxide and carbonate bound copper

The 0 Cu treatment demonstrated a clear increase in the amounts of copper held by these sites. The other treatments displayed a rapid fall in both amounts and percent extractable.

0.04M hydroxylammonium chloride in 25% acetic acid - Fe and Mn oxide bound copper
For all treatments, copper levels in this fraction of sites remained steady for the first 21 days after which there was an increase in both the amounts and percentages bound. For the 0 mg treatment there was a large increase between 179 and 238 days (21.1 $mgkg^{-1}$ to 111.8 $mgkg^{-1}$). There was also an increase in extractable copper for the 50 mg copper treatment (from 326.75 $mgkg^{-1}$ to 363.74 $mgkg^{-1}$) but the increase was much less marked - an increase by only 2.56% as opposed to 17.05%. For the higher copper concentrations there were decreases in the amounts bound.

0.1M EDTA - carbonate occluded/chelate extractable copper
For most treatments there was an initial increase and then a fall after 179 days in levels of copper bound by these sites. Only at the highest treatment level (150mg treatment) was there no drop in the amount of copper bound.

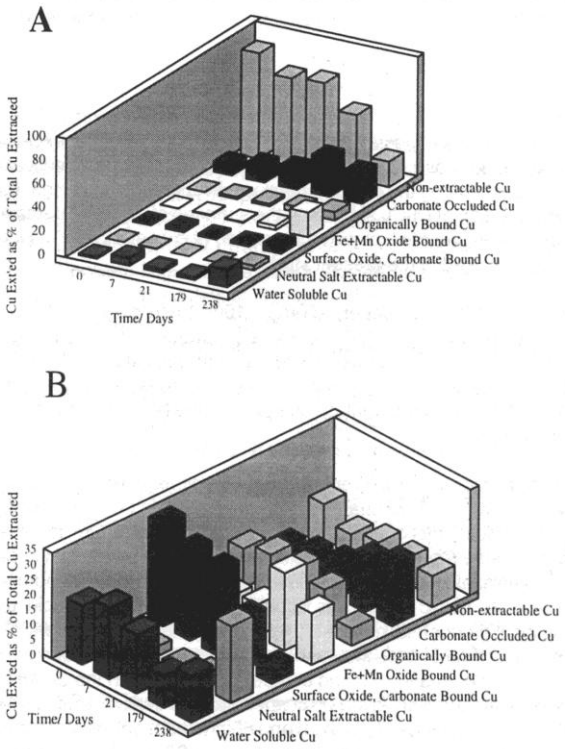

Fig. 1. Partitioning of copper during composting: A. 0 mg copper (control)
B. 150 mg copper / 100g fresh material

Residual - non-extractable copper

Residual levels for the 0mg, 100mg and 150mg treatments were similar at the start of the experiment indicating that copper moved very slowly from these sites. The 50mg treatment had very low levels extractable initially. When no copper was added there was a gradual reduction in the copper bound in non-extractable forms. Native copper is gradually released over time to the more available fractions. The same was true for the 50mg treatment for day 7 and later. For the two higher concentrations there was an overall decrease over time in the amounts bound but the processes were much slower.

Total Copper

Percentage copper recovered was very high in nearly all treatments and frequently exceeds 130%. This was likely to be due to incomplete digestion of the whole material and may have been the reason for there being no clear demonstration (with the exception of the 0 mg treatment) of the concentrating of heavy metals by the composting process. As standards for the AAS were prepared to be as closely matrixed matched as possible there was no reason to suppose that these anomolies resulted from spectral interferences.

Discussion

In treatments where copper had been added, concentrations at the start of the experiment in all fractions but the 'residual' far exceeded those of the system where no copper had been added indicating that copper was quickly distributed among the different fractions. Workers have demonstrated that maximum adsorption of copper in a solution of $CuCl_2.2H_2O$ onto the surfaces of sawdust particles is reached within an hour of the material coming into contact with the solution (Ajmal et al, 1998). However the salt used here was different to the one used in the present study.

Even with such high levels of added copper, 150mg / 100g fresh material (1500 $mgkg^{1}$), the cation exchange capacity of the composting material was not saturated. This is not surprising given that an application to the system of 150mg of copper would have an equivalency of only 4.7meq/100g fresh material. Cation exchange capacities have been found to increase over the composting period as humification proceeds and capacities of mature composts have been found to vary from 36.9 to 228.6 meq / 100g (Estrada et al 1987).

Copper was re-distributed among the different chemical fractions over the course of the composting period. Some of this was native to the substrates being composted and was released gradually over time from the tightly bound residual fraction. This was observed in the 0 Cu treatment. As composting proceeded copper then became more easily extracted by EDTA and potassium di-phosphate solutions, probably as a result of complexation of the copper with ligands formed during humification. As compost matured, increasingly aromatic humic substances would be formed (with high molecular weight and many oxygen-containing functional groups), all necessary for high-affinity metal binding (Petruzzelli 1996). As the copper load within this portion of sites increased, or perhaps as a result of a change in pH, then the Fe and Mn oxide sites played a greater role and competed strongly with the organic ligands for copper. These oxide sites also appeared to start receiving copper from the sodium acetate (the surface oxide and carbonates) extractable fraction. It was only in the 0 and 50mg treatments that the quantity of copper held by these sites continued to increase between days 179 and 238. At higher concentrations there were marked reductions between these two points over time. Sites binding copper extractable by sodium acetate released copper over time. Some of these ions may have moved into solution but most of them in the higher copper treatments will have moved into the more strongly bound fractions.

Concentrations of copper in binding sites represented by fractions 3-7 reached equilibrium. This was characterized by very similar percentages of copper bound over the whole the composting period. As this point was reached, the calcium nitrate and water extractable sites received the copper released.

Speciation assessments are dependent upon the initial starting materials (Petruzzelli 1996). Research into the speciation of copper in 1 and 3 month-old MSW compost has been conducted (Prudent et al 1993) and it was shown that just as with the starting materials used in the 0 mg treatment of the grass / sawdust blend study, the majority of the native copper is bound within the residual fraction. These workers also found that a significant portion of the copper in the MSW composts was to be found in the elemental form in small-sized particles which, when dissolved were taken up by the residual fraction, immobilizing the metal and increasing the percent of copper held here. As far as we know, such particles of elementary copper were not present in the grass/sawdust study and indeed the system demonstrated an overall release from the residual fraction.

Metals extracted from the MSW compost under reducing conditions such as with the hydroxylammonium chloride in acetic acid were found to be insignificant. Concentrations of copper extracted by this solvent in the grass/sawdust blend composts were very low for the 0 mg Cu treatment but increased sharply towards the end of the composting period. Such differences in behaviour have to be accounted for by differences in the starting materials used. Concentrations of native copper were already high (in the order of 500 $mgkg^{-1}$), which may be due to pre-treatment of the wood with copper-chromate-arsenate (CCA). This is applied under pressure to timber used in joists and floorboards as an insecticide to prevent infestation by pests such as the woodworm. The use of CCA is common practise within the timber industry.

Availability of metals bound to humic substances are generally considered to be low and therefore pose no (or negligible) threat to the environment. However, interactions between humic substances and metal ions are so complex and the bonding strengths so easily modified by pH, Eh and humification that this cannot always be assumed (Massiani, C and Domeizel M. 1996). In the present study, release from the organic and residual fractions to those more often identified as being phytoavailable has been demonstrated.

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Appendix II

Raw AAS data were converted to levels of extracted copper using Microsoft® Excel.

Extraction tubes and their contents were weighed after decantation of extracts and water washes so that any carry over of copper between extracts could be accounted for. The spreadsheet as given in Figure 1 was created for the first extract (water extract in Chapter 2; 0.11M Acetic Acid in the BCR scheme and 1M Magnesium Chloride).

A separate spreadsheet was created for all subsequent extracts and any copper present in the water wash left in the sample tube after the previous extraction was subtracted from the copper extracted by the currant extractant. In most cases however, this was negligible. The sheet created for the calcium nitrate extracts as used in Chapter 2 is given in Figure 2 as an example. The spreadsheet used for the residual digests is given in Figure 3.

It was assumed that the sample was not consumed during the extraction process until it was digested during the final residual phase. Calculations employed in the spreadsheets were as follows:

Calculations For the First Extraction

Spreadsheet

H. $$\text{Cu Extracted from Sample (mg)} = \frac{\text{Cu in Extract (mg/l)}}{1000} \times \text{Extractant Volume (ml)}$$

I. $$\text{Cu (Water Extractable) in Compost (mg/kg)} = \text{Cu Extracted from Sample (mg)} \times \frac{1000}{\text{Sample Mass (g)}}$$

F. $$\text{Volume of Extract Left Behind (ml)} = \text{Mass Tube and Lid After H2O Extract Removed (g)} - (\text{Mass Tube and Lid} + \text{Sample Mass (g)})$$

J. $$\text{Cu in the Extract Left Behind (mg)} = \frac{\text{Cu in Extract (mg/l)}}{1000} \times \text{Volume of Extract Left Behind (ml)}$$

L. $$\text{Volume Water Wash Left Behind} = \text{Mass Tube and Lid After H2O Wash} - (\text{Mass Tube and Lid} + \text{Sample Mass (g)})$$

Figure 1. Spreadsheet No.1 for Calculations Relating to the Copper Extracted in the Water Extracts. Text in RED gives the equations used with capital letters referring to the columns.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Tube No.	Tube+lid/g	Sample	Sample/g	Tube+lid after H20 extract/g	Volume Extract left behind ml	Cu in Extract mg/l	Cu extracted from Sample mg	Cu Extracted from Sample mg/kg	Cu in extract left behind mg	Tube+lid after H20 wash/g	Volume Wash left behind ml	Cu in wash left behind mg
2													
3													
4													
5													
6						=E-(B+D)	Raw AAS Data	=(G/1000)*30	=H*(1000/D)	=(G/1000)*F		=K-(B+D)	=J/((F+20)/L))
7													
8													
9													
10	1	23.832	0Cua 1	1.999	33.76	7.929	0.4299	0.012897	6.45172586	0.00340868	34.115	8.284	0.001011045
11	2	23.799	0Cua 2	1.999	34.808	9.01	0.446	0.01338	6.69334667	0.00401846	34.236	8.438	0.00116883
12	3	24.143	0Cua 3	2.001	34.598	8.454	0.4427	0.013281	6.63718141	0.00374259	34.828	8.684	0.001142216
13	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.
16	34	24.081	50Cue 2	2.001	33.445	7.363	15.13	0.4539	226.836582	0.11140219	33.533	7.451	0.030335041
17	35	23.834	50Cue 3	1.999	33.659	7.826	14.89	0.4467	223.461731	0.11652914	34.432	8.599	0.036010712

Figure 2. Spreadsheet No.2 for Calculations Relating to the Copper Extracted in the Calcium Nitrate Extracts. Text in RED gives the equations used with capital letters referring to the columns.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Tube No.	Tube+lid/g	Sample	Sample/g	Tube and lid after calcium nitrate extract/g	Volume Extract left behind ml	Cu in Extract mg/l	Cu extracted From Sample mg	Cu Extracted from Sample mg/kg	Cu in extract left behind mg	Tube+lid after H20 wash/g	Volume Wash left behind ml	Cu in wash left behind mg
2													
3													
4													
5						=E-(B+D)	Raw AAS Data	=(G/1000)*(30+Sheet1!L)	=(H-Sheet1!M)*(1000/D)	=(G/1000)*F		=K-(B+D)	=J/((F+20)/L))
6													
7													
8	1	23.832	0Cua 1	1.999	34.47	8.639	0	-0.001011045	-1.011550965	0	34.406	8.575	0
9	2	23.799	0Cua 2	1.999	34.707	8.909	0	-0.00116883	-1.169414955	0	34.287	8.489	0
10	3	24.143	0Cua 3	2.001	35.244	9.1	0.0173	-0.000472983	-0.807195838	0.00015743	35.342	9.198	4.97609E-05
11	.	.	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.
14	34	24.081	50Cue 2	2.001	35.859	9.777	2.975	0.081081684	25.36064152	0.02908658	36.457	10.375	0.01013444
15	35	23.834	50Cue 3	1.999	35.266	9.433	2.994	0.079554694	21.78288234	0.0282424	34.949	9.116	0.008747248

Figure 3. Spreadsheet No.7 for Calculations Relating to the Copper Extracted During the Digestion of the Residual Materials. Text in RED gives the equations used with capital letters referring to the columns

	A	B	C	D	E	F	G
1	Tube No.	Sample	Sample/g	Cu in Digestate mg/l	Residual Cu in Sample mg	Residual Copper in Compost mg/kg	
2							
3							
4							
5					=(D/1000)*50	=(E-EDTA!L)*(1000/C)	
6							
7	1	0Cua 1	1.999	15.18	0.751584267	375.9801235	
8	2	0Cua 2	1.999	20.13	1.002234605	501.3679865	
9	3	0Cua 3	2.001	17.11	0.851346015	425.4602771	
10	.	.	.	.	.	.	
11	.	.	.	.	.	.	
12	.	.	.	.	.	.	
13	34	50Cue 2	2.001	14.83	0.713702474	356.6729005	
14	35	50Cue 3	1.999	sample lost	*	*	

M. Copper in the Water Wash Left Behind (mg) = Cu in the Extract Left Behind / (Vol. Extract Left Behind + Vol. Water Wash / Vol. Water Wash Left Behind)

Calculations For all Subsequent Extractions

These equations were the same as for the water extracts, with the exceptions of the first two equations which took into account any copper remaining in the sample after the previous water wash and also the volume of that water wash which had been left behind. Using the Calcium Nitrate Extract as an example, these equations were now:

Spreadsheet

Column

H. Cu Extracted From Sample (mg) = Cu in Extract (mg/l) / 1000 x (Extractant vol. + Vol. Water Wash Left Behind)

I. Cu (CaNO3 Extractable) in Compost = Cu Extracted from Sample (mg) - Copper in the Water Wash Left Behind (mg) x 1000 / Sample Mass (g)

Calculations For the Residual Digestion

50ml of digestate were prepared for the scheme used in Chapter 2 and 100ml were prepared for the BCR and Tessier schemes used in Chapter 3.

The equations used were as follows:

Spreadsheet

Column

E. Residual Cu in Sample (mg) = $\frac{\text{Cu in Digestate (mg/l)}}{1000}$ x Digestate Volume (ml)

F. Residual Copper in Compost (mg/kg) = (Residual Cu in Sample - Cu in Water Wash Left Behind) x $\frac{1000}{\text{Sample Mass (g)}}$

.

Appendix III

Distribution of % Relative Standard Deviation Values of all samples for Copper Extracted (mg kg^{-1})

%RSD Ranges	Frequency of values	Percent Occurrence of Frequencies	Cumulative Frequencies	Frequency as % of cumulative frequencies
<0	2.00	1.43	2.00	1.43
0-0.999	4.00	2.86	6.00	4.29
1 - 1.999	8.00	5.71	14.00	10.00
2 - 2.999	14.00	10.00	28.00	20.00
3 - 3.999	4.00	2.86	32.00	22.86
4 - 4.999	7.00	5.00	39.00	27.86
5 - 5.999	7.00	5.00	46.00	32.86
6 - 6.999	15.00	10.71	61.00	43.57
7 - 7.999	5.00	3.57	66.00	47.14
8 - 8.999	5.00	3.57	71.00	50.71
9 - 9.999	5.00	3.57	76.00	54.29
10 - 10.999	8.00	5.71	84.00	60.00
11 - 11.999	5.00	3.57	89.00	63.57
12 - 12.999	6.00	4.29	95.00	67.86
13 - 13.999	6.00	4.29	101.00	72.14
14 - 14.999	5.00	3.57	106.00	75.71
15 - 15.999	1.00	0.71	107.00	76.43
16 - 16.999	3.00	2.14	110.00	78.57
17 - 17.999	2.00	1.43	112.00	80.00
18 - 18.999	4.00	2.86	116.00	82.86
19 - 19.999	3.00	2.14	119.00	85.00
20 - 20.999	4.00	2.86	123.00	87.86
21 - 21.999	2.00	1.43	125.00	89.29
22 - 22.999	2.00	1.43	127.00	90.71
23 - 23.999	2.00	1.43	129.00	92.14
24 - 24.999	0.00	0.00	129.00	92.14
25 - 25.999	0.00	0.00	129.00	92.14
26 - 26.999	0.00	0.00	129.00	92.14
27 - 27.999	0.00	0.00	129.00	92.14
28 - 28.999	2.00	1.43	131.00	93.57
29 - 29.999	0.00	0.00	131.00	93.57
30 - 30.999	0.00	0.00	131.00	93.57
31 - 31.999	0.00	0.00	131.00	93.57
32 - 32.999	1.00	0.71	132.00	94.29
33 - 33.999	0.00	0.00	132.00	94.29
34 - 34.999	0.00	0.00	132.00	94.29
35 - 35.999	0.00	0.00	132.00	94.29
36 - 36.999	0.00	0.00	132.00	94.29
37 - 37.999	0.00	0.00	132.00	94.29
38 - 38.999	0.00	0.00	132.00	94.29
39 - 39.999	3.00	2.14	135.00	96.43
47 - 47.999	1.00	0.71	136.00	97.14
77 - 77.999	1.00	0.71	137.00	97.86
90 - 90.999	1.00	0.71	138.00	98.57
91 - 91.999	1.00	0.71	139.00	99.29
153 - 153.999	1.00	0.71	140.00	100.00

Appendix IV

BCR Extraction Sequential Extraction Scheme (Ure *et. al.* 1993)

All samples were measured in duplicate. Material (2.000 ± 0.005g) was weighed out into 50ml polysulphone screw-capped tubes (Nalgene^{TM}, oak ridge with sealing caps as per Fraser and Lum, 1983) and subjected to the sequential extraction scheme as outlined in Table 1. The extractant was removed at the end of each stage using a Pasteur pipette and stored at <5˚C prior to analysis. The residue remaining at the end of each step was washed as per the original protocol by shaking with 20ml deionised water for 15 minutes and centrifuging. A Pasteur pipette was used to remove the supernatant which was then discarded, taking care to avoid losses of material. After the removal of each extractant and water wash the weights of the tubes were recorded in order to calculate the volume of reagent or water wash left behind. Copper in each extract was then determined by atomic absorption spectrophotometry at 324.7nm on a Smith Heiftje 1000 Atomic Absorption Spectrophotometer using matrix matched standards.

All reagents were Analar grade, or better, and all glassware was pre-soaked overnight in 5% HNO_3 and then rinsed 5 times in deionised water.

Tessier *et. al.* (1979) Sequential Extraction Scheme

All samples were analysed for copper using the scheme devised by Tessier *et. al.* (1979). This scheme was chosen as it yields more data than the BCR

Table 1 BCR Extraction Scheme (Ure *et. al.* 1993) with Additional Residual Digestion

Stage	Fraction	Reagent	Shaking time and termperature
1	Extractable	40ml 0.11M Acetic acid	16 hours, room temperature
2	Easily Reducible	40ml 0.1M Hydroxylammonium chloride (acidified to pH 2.0 with c.HNO_3)	16 hours, room temperature
3	Organic	10 ml 8.8M H_2O_2 10 ml 8.8M H_2O_2 50ml 1M Ammonium acetate (acidified to pH 2 with c.HNO_3)	1 hour, covered at room temperature with occasional manual shaking; 1 hour covered with watch glass at 85^oC; Uncovered at 85^oC to reduce to a few ml. 1 hour covered with watch glass at 85^oC; Uncovered at 85^oC to reduce to a few ml. Cool. 16 hours, room temperature
4	Residual	30ml *aqua regia*	Transfer to 500ml Quickfit conical flask, dry at 100^oC, then heat gently on hotplate for 3 hours under reflux using cold-finger condensers. Cool, then filter via Whatman 541 filter paper and dilute to 100ml with deionised water.

Table 2 Tessier *et. al.* (1979) Sequential Extraction Scheme

Stage	Fraction	Reagent	Shaking time and temperature
1	Exchangeable	8 ml of $MgCl_2$ 1mol dm^{-3} (pH = 7)	1hr at 25°C
2	Carbonate-bound	25 ml of NaOAc 1 mol dm^{-3} (pH = 5)	5 h at 25°C
3	Fe-Mn oxides	20 ml $NH_2OH.HCl$ 0.04 mol dm^{-3} in HOAc 25% m/m	6 h at 96°C
4	Organic matter	3 ml HNO_3 0.02 mol dm^{-3} + 5 ml H_2O_2 30% m/v 3 ml H_2O_2 30% m/v 5 ml NH_4OAc 3.2 mol dm^{-3} in 20% HNO_3	2 h at 85°C 3 h at 85°C 30 min at 25°C
5	Residual	1 ml HCl 35% m/m + 2 ml HF 48% m/m+ 4 ml HNO_3 70% m/m + 5 ml H_2O	26 min

scheme, is the one most often used and is considered to be the more classical approach. Details of the original scheme are given in Table 2.

2g ± 0.001g each of the ground samples were weighed out into 50ml polyethylene tubes (Sarstedt) and subjected to the sequential analysis scheme as detailed in Table 2, but modified with respect to stage five. Here the original step was replaced with Method 3050b (Office of Solid Waste, 1996) which involved digestion with a strong acid but does not use HF. Tubes containing the sample and extractant were shaken on a reciprocal shaker for the required period of time, with the exception of stage 3 in which they were placed in a water bath and occasionally agitated by hand. After shaking, the tubes were centrifuged at 15,000 rpm in a Beckman Avanti 30 centrifuge at 4°C for 30 minutes. The supernatants were transferred, using disposable Pasteur pipettes, into either 8ml or 25ml screw capped polyethylene tubes, depending on the volume of extractant. Filtration was not found to be necessary as use of the Pasteur pipettes meant that losses of material could be largely avoided. The pellets of material left after each extraction were

subjected to a water wash (20ml deionised water, shaking time 15 minutes, centrifugation at 15,000 rpm for 15minutes and removal by Pasteur pipette) which was then discarded. This was done in order that any residual extractant might be removed (Miller *et. al.* 1986). After the removal of every extractant or water wash the weights of the tubes were recorded in order to calculate the volume of reagent or water wash left behind. Extracts were acidified to pH<2.0 with concentrated HNO_3, with the exception of the sodium acetate extract which was too strongly buffered and had to be frozen instead.

For the determination of stage 5, the 'residual' copper, the pellets of material left in the tubes after the previous 4 extractions were quantitatively transferred to 250ml glass digestion tubes using deionised water administered via a wash bottle. Water was then removed by placing the tubes overnight in an oven at 105˚C. Once dried, the samples were digested according to Method 3050b (Office of Solid Waste, 1996) a strong acid digestion which determines total potential environmentally available copper. This measurement is not a measure of the absolute total amount present as copper bound within silicate structures is not dissolved by this technique (Office of Solid Waste, 1996). However, as elements retained within these structures are not usually mobile in the environment they can be disregarded for the purposes of this research.

All reagents were Analar grade, or better, and all glassware was pre-soaked overnight in 5% HNO_3 and then rinsed 5 times in deionised water. Analyses were performed in triplicate. Copper in each extract was determined on a

Smith-Hieftje 1000 Atomic Absorption Spectrophotometer at 324.7nm calibrated using matrix-matched samples.

Method 3050b (Office of Solid Waste, 1996)

This procedure is a strong acid digestion for the determination of total potentially environmentally available copper:

To the dried material in the digestion tubes was added 10ml of 1:1 (acid : water) HNO_3 and the tubes shaken gently to mix the slurry. All the tubes were then heated to 95˚C under reflux (by placing a watch glass over the mouth of each tube), and without boiling, on an aluminium heating block for 15 minutes and then cooled. A further 5ml of 1:1 HNO_3 were added to each tube and the watch glasses replaced and the tubes heated again under reflux for 30 minutes. Successive 5ml aliquots of 1:1 HNO_3 were added until no more brown fumes were generated (brown fumes indicate oxidation of the sample by the acid). Tubes were then heated at 95˚C for 2 hours and then cooled. Next, 2ml of deionised water were added and then, very carefully because of vigorous effervescence, 3ml of 30% H_2O_2. Further 1ml aliquots of H_2O_2 were added (up to a maximum of 10ml as per the method), with warming, until either the effervescence had stopped or the appearance of the sample had ceased to change. It was noted that all samples required the full 10ml of H_2O_2. Tubes were then heated until the volume of the digestate had reduced to approximately 5ml. 10ml of concentrated HCl were then added to each

digestate in their tubes. Each tube was then covered with a watch glass and heated under reflux on the heating block at 95˚C for 15 minutes. Once cooled, the digestates were filtered via a Whatman No. 41 filter paper into a 100ml volumetric flask and diluted to volume with deionised water. The contents of the flasks were thoroughly mixed and 50ml retained and stored in polypropylene containers for subsequent analysis of copper by flame AAS.

Appendix V

Tessier Extractions – MgCl2 Extracts

- Grass blends
- 0 Days
- mg/kg

Analysis of variance

Variate: MgCl2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	7696.	3848.	0.38	
Block.*Units* stratum					
Treatment	3	4921254.	1640418.	160.29	<.001
Residual	6	61406.	10234.		
Total	11	4990355.			

Tables of means

Variate: MgCl2

Grand mean 1014.

Treatment	1	2	3	4
	0.	954.	1423.	1679.

Standard errors of means

Table	Treatment
rep.	3
d.f.	6
e.s.e.	58.4

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	6
s.e.d.	82.6

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	6

l.s.d. 202.1

Stratum standard errors and coefficients of variation

Variate: MgCl2

Stratum	d.f.	s.e.	cv%
Block	2	31.0	3.1
Block.*Units*	6	101.2	10.0

All pairwise comparisons are tested.

Variance = 10234.2531 with 6 degrees of freedom

Tukey's 95 % confidence intervals

Mean	- Mean	Lower	Difference	Upper	significant
1	2	-1240.1644	-954.2241	-668.2838	Yes
1	3	-1708.4311	-1422.4908	-1136.5504	Yes
1	4	-1964.6089	-1678.6685	-1392.7282	Yes
2	3	-754.2070	-468.2667	-182.3263	Yes
2	4	-1010.3848	-724.4444	-438.5041	Yes
3	4	-542.1181	-256.1778	29.7626	No

Identifier	Mean	
1	0	
2	954	
3	1423	|
4	1679	|

Appendix VI

Analysis of Data from Albores *et al* 2000

Total Metals Extracted by Tessier and BCR Schemes

	Tessier			BCR					
	Amount Extracted mg/kg	SD	SE squared	Amount Extracted mg/kg	SD	SE squared	Calculated T-Value	Bigger than critical t? (Critical t=2.78)	Scheme Extracting Largest Amount
Sample A									
Cu	315.5	4.2	5.88	291.9	1.6	0.853333333	9.0948794	Y	Tessier
Cr	5.14	0.22	0.016133333	7.79	0.39	0.0507	10.250601	Y	BCR
Ni	11.95	0.35	0.040833333	10.82	0.69	0.1587	2.5297099	N	N/A
Pb	54.24	0.78	0.2028	15.12	0.34	0.038533333	79.632471	Y	Tessier
Zn	679.9	9.6	30.72	573.2	3.6	4.32	18.025295	Y	Tessier
Sample B									
Cu	538.1	0.7	0.163333333	392.6	8.7	25.23	28.873746	Y	Tessier
Cr	6.7	0.18	0.0108	9.38	0.74	0.182533333	6.0951083	Y	BCR
Ni	15.28	0.55	0.100833333	10.76	0.45	0.0675	11.016747	Y	Tessier
Pb	32.76	1.04	0.360533333	1.62	0.1	0.003333333	51.623503	Y	Tessier
Zn	771.9	4.34	6.278533333	705.3	15	75	7.3873096	Y	Tessier

Fraction 1 (where BCR is assumed to = F1 and F2 of Tessier)

	Tessier			BCR					
	Amount Extracted mg/kg	SD	SE squared	Amount Extracted mg/kg	SD	SE squared	Calculated T-Value	Bigger than critical t? (Critical t=2.78)	Scheme Extracting Largest Amount
Sample A									
Cu	26.74	0.23	0.017633333	22.01	0.58	0.112133333	13.130449	Y	Tessier
Cr	nd	nd	nd	nd	nd	nd	nd	N/A	N/A
Ni	3.98	0.18	0.0108	2.89	0.22	0.016133333	6.6417335	Y	Tessier
Pb	nd	nd	nd	nd	nd	nd	nd	N/A	N/A
Zn	106.97	0.68	0.154133333	34.12	0.69	0.1587	130.24859	Y	Tessier
Sample B									
Cu	29.26	0.18	0.0108	25.77	0.32	0.034133333	16.464218	Y	Tessier
Cr	nd	nd	nd	nd	nd	nd	nd	N/A	N/A
Ni	4.89	0.1	0.003333333	2.9	0.21	0.0147	14.818869	Y	Tessier
Pb	9.09	0.35	0.040833333	0	0	0	44.983834	Y	Tessier
Zn	94.08	1.37	0.625633333	40.33	1.04	0.360533333	54.125673	Y	Tessier

Fraction 2 - reducible

	Tessier			BCR					
	Amount Extracted mg/kg	SD	SE squared	Amount Extracted mg/kg	SD	SE squared	Calculated T-Value	Bigger than critical t? (Critical t=2.78)	Scheme Extracting Largest Amount
Sample A									
Cu	2.12	0.16	0.008533333	0	0	0	22.949673	Y	Tessier
Cr	1.79	0.11	0.004033333	0	0	0	28.18519	Y	Tessier
Ni	4.04	0	0	0	0	0	-	Y	Tessier
Pb	41.69	0.45	0.0675	0	0	0	160.46488	Y	Tessier
Zn	488.1	9.5	30.08333333	64.39	0.82	0.224133333	76.96511	Y	Tessier
Sample B									
Cu	12.62	0.57	0.1083	0	0	0	38.348213	Y	Tessier
Cr	nd	nd	nd	nd	nd	nd	nd	N/A	N/A
Ni	3.28	0.24	0.0192	0	0	0	23.671361	Y	Tessier
Pb	22.12	0.92	0.282133333	0	0	0	41.644526	Y	Tessier
Zn	546.7	2.5	2.083333333	47.43	1.17	0.4563	313.29247	Y	Tessier

Fraction 3 - oxidisable

	Tessier			BCR					
	Amount Extracted mg/kg	SD	SE squared	Amount Extracted mg/kg	SD	SE squared	Calculated T-Value	Bigger than critical t? (Critical t=2.78)	Scheme Extracting Largest Amount
Sample A									
Cu	286.6	4.3	6.163333333	269.9	1.5	0.75	6.3514476	Y	Tessier
Cr	3.35	0.21	0.0147	7.79	0.39	0.0507	17.361774	Y	BCR
Ni	3.93	0.33	0.0363	7.93	0.65	0.140833333	9.5040748	Y	BCR
Pb	9.49	0.6	0.12	15.12	0.34	0.038533333	14.139957	Y	BCR
Zn	84.83	1	0.333333333	474.7	3.4	3.853333333	190.53977	Y	BCR
Sample B									
Cu	396.2	0.3	0.03	366.8	8.74	25.46253333	5.8229201	Y	Tessier
Cr	6.7	0.18	0.0108	9.38	0.74	0.182533333	6.0951083	Y	BCR
Ni	7.11	0.49	0.080033333	7.86	0.4	0.053333333	2.0537029	N	N/A
Pb	1.55	0.12	0.0048	1.62	0.43	0.061633333	0.2715845	N	N/A
Zn	131.2	3.4	3.853333333	617.5	14.9	74.00333333	55.113294	Y	BCR

Appendix VII

FTIR Data Analysis

Identical superscript lettering indicates non-significance at 95% confidence limits

Table 1 T = 0 Days, Grass/Sawdust Blend. Absorbance Values of Selected Wavenumbers

Wavenumber (nm)	Treatment			
	1	2	3	4
1030	0.783 [a,b,c,d,e,f]	0.834 [a,b,c,d,e,f]	0.830 [a,b,c,d,e,f]	0.952 [e,f]
1100	0.730 [a,b,c,d,e,f]	0.771 [a,b,c,d,e,f]	0.761 [a,b,c,d,e,f]	0.891 [b,c,d,e,f]
1240	0.558 [a,b]	0.581 [a,b]	0.551 [a,b]	0.694 [a,b,c,d,e,f]
1320	0.538 [a]	0.559 [a,b]	0.529 [a]	0.677 [a,b,c,d,e,f]
1430	0.539 [a]	0.562 [a,b]	0.529 [a]	0.679 [a,b,c,d,e,f]
1643	0.567 [a,b]	0.599 [a,b,c,d]	0.560 [a,b]	0.720 [a,b,c,d,e,f]
2850	0.580 [a,b]	0.560 [a,b]	0.516 [a]	0.636 [a,b,c,d,e]
2920	0.659 [a,b,c,d,e,f]	0.630 [a,b,c,d,e]	0.590 [a,b,c]	0.702 [a,b,c,d,e,f]
3400	0.933 [d,e,f]	0.940 [d,e,f]	0.932 [c,d,e,f]	1.00 [f]

Table 2 T = 105 Days, Grass/Sawdust Blend. Absorbance Values of Selected Wavenumbers.

Wavenumber (nm)	Treatment			
	1	2	3	4
1030	0.768 [a,b,c,d,e,f,g]	0.882 [c,d,e,f,g]	0.863 [b,c,d,e,f,g]	0.857 [b,c,d,e,f,g]
1100	0.722 [a,b,c,d,e,f,g]	0.769 [a,b,c,d,e,f,g]	0.784 [a,b,c,d,e,f,g]	0.758 [a,b,c,d,e,f,g]
1240	0.566 [a,b,c,d,e]	0.544 [a,b,c]	0.575 [a,b,c,d,e]	0.536 [a,b]
1320	0.558 [a,b,c,d]	0.545 [a,b,c]	0.575 [a,b,c,d,e]	0.539 [a,b]
1430	0.558 [a,b,c,d]	0.538 [a,b]	0.564 [a,b,c,d,e]	0.528 [a,b]
1643	0.583 [a,b,c,d,e]	0.555 [a,b,c]	0.583 [a,b,c,d,e]	0.578 [a,b,c,d,e]
2850	0.550 [a,b,c]	0.499 [a]	0.538 [a,b]	0.502 [a]
2920	0.618 [a,b,c,d,e,f]	0.576 [a,b,c,d,e]	0.607 [a,b,c,d,e,f]	0.577 [a,b,c,d,e]
3400	0.896 [d,e,f,g]	0.940 [f,g]	0.902 [e,f,g]	0.987 [g]

Table 3 T = 318 Days, Grass/Sawdust Blend. Absorbance Values of Selected Wavenumbers.

Wavenumber (nm)		Treatment			
		1	2	3	4
1030	1	0.756 [a]	0.727 [a]	0.791 [a]	0.809 [a]
1100	2	0.678 [a]	0.668 [a]	0.712 [a]	0.725 [a]
1240	3	0.457 [a]	0.498 [a]	0.511 [a]	0.533 [a]
1320	4	0.462 [a]	0.503 [a]	0.521 [a]	0.542 [a]
1430	5	0.455 [a]	0.501 [a]	0.509 [a]	0.531 [a]
1643	6	0.486 [a]	0.530 [a]	0.539 [a]	0.580 [a]
2850	7	0.461 [a]	0.521 [a]	0.522 [a]	0.540 [a]
2920	8	0.536 [a]	0.581 [a]	0.583 [a]	0.601 [a]
3400	9	0.911 [a]	0.905 [a]	0.944 [a]	0.963 [a]

Table 4 T = 0 Days, All Three Blends, Unamended and Copper Acetate Treated. Absorbance Values of Selected Wavenumbers

Wavenumber (nm)	Blend					
	UGS	AGS	UPS	APS	UCS	ACS
	1,1	1,2	2,1	2,2	3,1	3,2
1030	0.809[a]	0.834[a]	0.793[a]	0.905[a]	0.958[a]	0.864[a]
1100	0.756[a]	0.771[a]	0.718[a]	0.812[a]	0.861[a]	0.790[a]
1240	0.584[a]	0.581[a]	0.533[a]	0.575[a]	0.589[a]	0.574[a]
1320	0.563[a]	0.559[a]	0.521[a]	0.556[a]	0.550[a]	0.548[a]
1430	0.565[a]	0.562[a]	0.567[a]	0.600[a]	0.579[a]	0.565[a]
1643	0.592[a]	0.599[a]	0.572[a]	0.597[a]	0.611[a]	0.586[a]
2850	0.606[a]	0.560[a]	0.676[a]	0.661[a]	0.557[a]	0.583[a]
2920	0.684[a]	0.630[a]	0.760[a]	0.762[a]	0.654[a]	0.664[a]
3400	0.961[a]	0.940[a]	0.963[a]	0.986[a]	0.965[a]	0.969[a]

Table 5 T = 105 Days, All Three Blends, Unamended and Copper Acetate Treated. Absorbance Values of Selected Wavenumbers

Wavenumber (nm)	1		2		3	
	1	2	1	2	1	2
1030	0.854[a,b,c,d]	0.882[a,b,c,d]	0.753[a,b,c,d]	0.974[b,c,d]	0.819[a,b,c,d]	0.944[a,b,c,d]
1100	0.807[a,b,c,d]	0.769[a,b,c,d]	0.716[a,b,c,d]	0.871[a,b,c,d]	0.747[a,b,c,d]	0.841[a,b,c,d]
1240	0.651[a,b,c,d]	0.544[a,b]	0.553[a,b]	0.577[a,b,c]	0.544[a,b]	0.558[a,b]
1320	0.643[a,b,c,d]	0.545[a,b]	0.564[a,b]	0.593[a,b,c]	0.547[a,b]	0.554[a,b]
1430	0.643[a,b,c,d]	0.538[a,b]	0.585[a,b,c]	0.624[a,b,c,d]	0.563[a,b]	0.582[a,b,c]
1643	0.668[a,b,c,d]	0.555[a,b]	0.628[a,b,c,d]	0.657[a,b,c,d]	0.570[a,b]	0.614[a,b,c,d]
2850	0.6352[a,b,c,d]	0.499[a]	0.657[a,b,c,d]	0.599[a,b,c]	0.581[a,b,c]	0.586[a,b,c]
2920	0.703[a,b,c,d]	0.576[a,b,c]	0.707[a,b,c,d]	0.683[a,b,c,d]	0.638[a,b,c,d]	0.666[a,b,c,d]
3400	0.982[b,c,d]	0.940[a,b,c,d]	0.983[b,c,d]	1.06[d]	0.979[b,c,d]	1.03[c,d]

Table 6 T = 318 Days, All Three Blends, Unamended and Copper Acetate Treated. Absorbance Values of Selected Wavenumbers

Wavenumber (nm)	1		2		3	
	1	2	1	2	1	2
1030	0.801[a,b,c,d,e]	0.727[a,b,c,d,e]	0.773[a,b,c,d,e]	0.637[a,b,c,d,e]	0.789[a,b,c,d,e]	0.590[a,b,c,d,e]
1100	0.720[a,b,c,d,e]	0.668[a,b,c,d,e]	0.703[a,b,c,d,e]	0.581[a,b,c,d,e]	0.736[a,b,c,d,e]	0.545[a,b,c,d]
1240	0.502[a,b,c]	0.498[a,b,c]	0.492[a,b,c]	0.402[a]	0.576[a,b,c,d,e]	0.402[a]
1320	0.507[a,b,c]	0.503[a,b,c]	0.508[a,b,c]	0.418[a]	0.578[a,b,c,d,e]	0.403[a]
1430	0.500[a,b,c]	0.501[a,b,c]	0.531[a,b,c]	0.444[a]	0.600[a,b,c,d,e]	0.427[a]
1643	0.531[a,b,c]	0.530[a,b,c]	0.563[a,b,c,d,e]	0.479[a,b]	0.621[a,b,c,d,e]	0.456[a,b]
2850	0.506[a,b,c]	0.521[a,b,c]	0.580[a,b,c,d,e]	0.503[a,b,c]	0.658[a,b,c,d,e]	0.490[a,b]
2920	0.581[a,b,c,d,e]	0.581[a,b,c,d,e]	0.639[a,b,c,d,e]	0.554[a,b,c,d]	0.699[a,b,c,d,e]	0.531[a,b,c]
3400	0.956[d,e]	0.905[c,d,e]	0.972[e]	0.862[b,c,d,e]	0.970[e]	0.798[a,b,c,d,e]

Appendix VIII

Spearman's Rank Correlation Matrix[a] for Extractable Copper and FTIR Transmission at Selected Wavenumbers

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.00												
2	0.433**	1.00											
3	0.488**	0.455**	1.00										
4	0.387*	0.953***	0.580***	1.00									
5	0.074	-0.375*	-0.040	-0.336*	1.00								
6	0.058	-0.417*	-0.051	-0.376*	0.990***	1.00							
7	0.036	-0.471**	-0.037	-0.411*	0.921***	0.959***	1.00						
8	-0.011	-0.468**	-0.034	-0.391*	0.904***	0.942***	0.991***	1.00					
9	0.012	-0.422*	-0.088	-0.382*	0.904***	0.938***	0.981***	0.978***	1.00				
10	-0.011	-0.470**	-0.075	-0.415*	0.895***	0.931***	0.980***	0.986***	0.991***	1.00			
11	-0.061	-0.367*	-0.191	-0.329	0.806***	0.838***	0.910***	0.919***	0.957***	0.942***	1.00		
12	-0.075	-0.387*	-0.212	-0.356*	0.844***	0.873***	0.925***	0.928***	0.966***	0.948***	0.993***	1.00	
13	-0.116	-0.435**	-0.163	-0.392*	0.900***	0.928***	0.941***	0.954***	0.957***	0.959***	0.914***	0.932***	1.00

Key to Factors

1 = Cu in Fraction 1 of Tessier Scheme (mg kg^{-1})
2 = Cu in Sum of Fractions 1-5 of Tessier Scheme (mg kg^{-1})
3 = CRM Adsorbable Copper (µg cm^{-2})
4 = Total Cu by XRF
5 = Absorbance at 1030 cm^{-1}
6 = Absorbance at 1100 cm^{-1}
7 = Absorbance at 1240 cm^{-1}
8 = Absorbance at 1320 cm^{-1}
9 = Absorbance at 1430 cm^{-1}
10 = Absorbance at 1643 cm^{-1}
11 = Absorbance at 2850 cm^{-1}
12 = Absorbance at 2920 cm^{-1}
13 = Absorbance at 3400 cm^{-1}

a: *, ** and *** equals p≤ 0.05, 0.01 and 0.001 respectively.

Appendix IX

Determination of Ammonium and Nitrate- Nitrogen in Water Extracts (MAFF, 1986)

10ml of each sample extract were dispensed into a 250ml test-tube, placed in the autoanalyser and steam distilled with 7ml of a magnesium oxide suspension (17g in 100ml) to release the ammonium. The distillate was trapped in an indicator solution containing 1% Boric acid, methyl red and bromocresol green indicators and titration was then performed against 0.1N HCl until a pink coloured end point was reached. Once complete, the test-tube was removed from the analyser, approximately 1g of Devarda's alloy was then added, the tube returned to the autoanalyser and steam distilled once more. The Devarda's alloy reduced any nitrate present to ammonium, which, owing to the presence of the magnesium oxide was then distilled, trapped in the indicator solution and titrated against in the same manner as before. 10ml of ammonium and nitrate standards containing 0.14mgN/ml were prepared and analysed to check for recovery with titre-blank results of 1.000ml being equal to 100% recovery. Average recovery of the machine was 96.5% for the ammonium and 98.2% for the nitrate when the t = 0 days samples were analysed and 98.38% and 99.1% for the ammonium and nitrate samples when the t = 318 days samples were analysed.

Closed Reflux, Colorimetric Method for the Determination of Chemical Oxygen Demand (APHA, 1985)

Materials:

A: Digestion solution: Add to about 500ml of distilled water, 10.216g $K_2Cr_2O_7$ (Analar grade or better), previously dried at 103˚C for 2 hours, 167ml concentrated H_2SO_4 and 33.3g of $HgSO_4$ (to prevent interference form chlorides). Dissolve, cool to room temperature and dilute to 1000ml.

B: Suphuric Acid Reagent (1% m/v): Add 5.5g Ag_2SO_4 (GPR or Analar grade) to 1kg of concentrated H_2SO_4. Let stand for 1 to 2 days to dissolve.

C: Potassium Hydrogen Phthalate (KHP) Stock Standard Solution: Lightly crush and dry to a constant weight approx. 2g of KHP. Dissolve 1.275g of the dried KHP in deionised water and dilute to 1000ml. This solution has a theoretical COD of 1500 µg O_2/ml.

D: Potassium Hydrogen Phthalate (KHP) Standard Solutions: Pipette 5, 10, 25,and 50ml of the 1500 µg O_2/ml standard into 100ml volumetric flasks and then dilute to volume with deionised water. This gives a range of standards containing 75,150, 375 and 750 µg O_2/ml.

E: 200 µg O_2/ml control standard: This is a commercial preparation available from VWR (Product Code:xxx).

Method

2.5ml of the standard solutions, the stock standard solution, 200 µg O_2/ml standard and the water extracts are pipetted into 16 x 100mm culture tubes (urea caps with PTFE liners) which have previously been washed in 20% H_2SO_4 to prevent contamination. 1.5ml of the digestion solution are added and then 3.5ml of the sulphuric acid by carefully running the acid down the inside of the vessel so an acid layer is formed under the sample-digestion solution layer. Tubes are tightly capped, inverted several times to mix completely, and then placed in a rack in an oven preheated to 150˚C for 2 hours to reflux. Samples are left to cool, then inverted twice after 20 minutes whilst still warm and then left to settle until they reach room temperature, thus allowing any solids present allowed to settle. Unopended tubes are placed within a suitable holder within a spectrophotometer and absorbance at 600nm measured.* A calibration curve is prepared using the results from the standards, against which the samples are measured.

* Scratches on the outside surface of the culture tubes meant that absorbance at 600nm could not be measured in the unopened tubes as per the original method. The method was therefore adapted by using transfer pipettes to dispense the solutions into microcuvettes before placing in the spectrophotometer

Appendix X

Table 1 Spearman's Rank Correlation Matrix for all Grass Treatments. Samples taken from t = 0 and 318 days. Figures in RED p≤ 0.001; Figures in GREEN p≤ 0.010, Figures in BLUE p≤ 0.050.

Factor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.258	1.00																		
3	0.269	0.368	1.00																	
4	0.193	0.468	0.947	1.00																
5	-0.358	0.403	0.485	0.574	1.00															
6	0.060	-0.594	-0.774	-0.824	-0.688	1.00														
7	-0.493	0.343	0.515	0.624	0.633	-0.796	1.00													
8	-0.734	0.173	0.291	0.388	0.547	-0.550	0.767	1.00												
9	-0.674	-0.288	-0.398	-0.423	0.178	0.188	-0.044	0.276	1.00											
10	-0.755	0.120	0.253	0.305	0.532	-0.507	0.677	0.947	0.499	1.00										
11	0.259	-0.456	-0.526	-0.626	-0.765	0.847	-0.655	-0.656	0.019	-0.581	1.00									
12	-0.304	-0.564	-0.337	-0.270	-0.149	0.447	0.028	-0.117	0.145	-0.152	0.484	1.00								
13	-0.325	0.685	0.262	0.350	0.382	-0.594	0.734	0.657	-0.103	0.567	-0.450	-0.229	1.00							
14	-0.615	0.479	0.121	0.221	0.553	-0.521	0.639	0.819	0.357	0.812	-0.582	-0.248	0.791	1.00						
15	0.243	0.909	0.247	0.306	0.288	-0.565	0.237	0.141	-0.178	0.144	-0.465	-0.674	0.624	0.518	1.00					
16	-0.708	0.176	0.306	0.424	0.515	-0.579	0.864	0.921	0.210	0.870	0.597	0.015	0.671	0.756	0.118	1.00				
17	-0.633	0.271	0.288	0.329	0.503	-0.615	0.761	0.836	0.153	0.791	-0.676	-0.302	0.694	0.726	0.279	0.856	1.00			
18	0.364	-0.279	-0.388	-0.388	-0.444	0.585	-0.552	-0.618	0.050	-0.558	0.662	0.480	-0.503	-0.453	-0.288	-0.576	-0.879	1.00		
19	-0.845	-0.091	0.124	0.165	0.506	-0.321	0.624	0.891	0.542	0.906	-0.462	0.119	0.444	0.741	-0.109	0.847	0.688	-0.368	1.00	
20	-0.422	0.274	0.594	0.676	0.694	-0.797	0.751	0.759	0.047	0.696	-0.874	-0.290	0.444	0.538	0.247	0.750	0.774	-0.771	0.571	1.00

Key to Factors:

1 = Cu in Fraction 1 of Tessier Scheme (mg kg^{-1})
2 = CRM Adsorbable Copper (µg cm^{-2})
3 = Cu in Sum of Fractions 1-5 of Tessier Scheme (mg kg^{-1})
4 = Total Cu by XRF
5 = % Organic Matter by Loss on Ignition
6 = pH of Water Extracts
7 = EC of Water Extracts (mS)
8 = NH_4^+ in Water Extracts (mg kg^{-1})
9 = NO_3^- in Water Extracts (mg kg^{-1})
10 = Sum of NH_4^+ and NO_3^- in Water Extracts (mg kg^{-1})
11 = COD (mg O_2 L^{-1})
12 = Germination Index
13 = Absorbance of Water Extracts at 280nm
14 = Absorbance of Water Extracts at 465 nm
15 = Absorbance of Water Extracts at 665 nm
16 = Total Organic Carbon (g/kg)
17 = Total Nitrogen as determined by Dumas method (g/kg)
18 = C/N Ratio (Factor 16/Factor 17)
19 = E4/E6 Ratio (Factor 14/Factor 16)
20 = Total Sulphur as Determined by Dumas Method

Table 2 Spearman's Rank Correlation Matrix for all Blends (unamended and copper acetate treated). Samples taken from t = 0 and 318 days. Figures in RED p≤ 0.001; Figures in GREEN p≤ 0.010, Figures in BLUE p≤ 0.050.

Factor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.839	1.00																		
3	0.722	0.683	1.00																	
4	0.645	0.587	0.949	1.00																
5	-0.105	0.098	-0.313	-0.274	1.00															
6	-0.348	-0.448	-0.145	-0.084	-0.414	1.00														
7	-0.236	0.132	-0.086	0.047	0.355	-0.008	1.00													
8	-0.066	-0.063	0.286	0.382	-0.094	0.662	0.209	1.00												
9	-0.107	0.033	-0.087	-0.028	0.160	0.231	0.479	0.220	1.00											
10	-0.063	-0.057	0.293	0.389	-0.093	0.652	0.213	0.993	0.273	1.00										
11	-0.038	-0.006	-0.450	-0.529	0.116	-0.631	-0.077	-0.872	-0.196	-0.864	1.00									
12	-0.152	-0.378	0.139	0.160	-0.811	0.431	-0.343	0.181	-0.193	0.186	-0.182	1.00								
13	-0.120	0.199	0.027	0.090	0.538	0.043	0.799	0.492	0.483	0.491	-0.315	-0.517	1.00							
14	-0.126	-0.010	0.340	0.379	0.014	0.435	0.242	0.826	0.267	0.825	-0.745	-0.025	0.572	1.00						
15	0.023	0.136	0.469	0.509	-0.130	0.546	0.226	0.753	0.363	0.760	-0.843	0.098	0.433	0.752	1.00					
16	0.023	-0.022	0.346	0.305	0.043	-0.134	-0.273	0.346	-0.298	0.337	-0.312	0.167	0.011	0.375	0.060	1.00				
17	-0.206	-0.143	0.195	0.288	-0.262	0.828	0.295	0.837	0.257	0.835	-0.818	0.293	0.380	0.687	0.807	0.048	1.00			
18	0.164	0.104	-0.185	-0.285	0.302	-0.816	-0.299	-0.764	-0.218	-0.756	0.764	-0.290	-0.328	-0.596	-0.799	0.111	-0.974	1.00		
19	-0.274	-0.082	-0.029	-0.036	0.283	0.075	0.264	0.426	0.040	0.422	-0.274	-0.319	0.540	0.687	0.137	0.448	0.248	-0.121	1.00	
20	-0.032	-0.031	0.403	0.497	-0.073	0.613	0.165	0.906	0.234	0.900	-0.928	0.214	0.425	0.784	0.881	0.302	0.857	-0.802	0.246	1.00

Key to Factors:

1 = Cu in Fraction 1 of Tessier Scheme (mg kg^{-1})
2 = CRM Adsorbable Copper (µg cm^{-2})
3 = Cu in Sum of Fractions 1-5 of Tessier Scheme (mg kg^{-1})
4 = Total Cu by XRF
5 = % Organic Matter by Loss on Ignition
6 = pH of Water Extracts
7 = EC of Water Extracts (mS)
8 = NH_4^+ in Water Extracts (mg kg^{-1})
9 = NO_3^- in Water Extracts (mg kg^{-1})
10 = Sum of NH_4^+ and NO_3^- in Water Extracts (mg kg^{-1})
11 = COD (mg O_2 L^{-1})
12 = Germination Index
13 = Absorbance of Water Extracts at 280nm
14 = Absorbance of Water Extracts at 465 nm
15 = Absorbance of Water Extracts at 665 nm
16 = Total Organic Carbon (mg kg^{-1})
17 = Total Nitrogen as determined by Dumas method (mg kg^{-1})
18 = C/N Ratio (Factor 16/Factor 17)
19 = E4/E6 Ratio (Factor 14/Factor 16)
20 = Total Sulphur as Determined by Dumas Method